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(54) Title: IMMUNOGENIC HIV PEPTIDES FOR USE AS REAGENTS AND VACCINES

(57) Abstract: Immunogenic HIV peptides and methods of use are provided in which each HIV peptide include epitopes that are immunoreactive with cytotoxic T lymphocytes (CTLs) from HIV-positive individuals and binds to antibodies that are immunoreactive with the assembled class I major histocompatibility complex (MHC) structure. Preferably, the peptide is an isolated or synthetic peptide containing between nine and eleven amino acid residues within specific regions of the HIV genome.

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## **IMMUNOGENIC HIV PEPTIDES FOR USE AS REAGENTS AND VACCINES**

This invention was made at the Centers for Disease Control and Prevention. Therefore, the United States Government has certain rights in this invention.

### **CROSS REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Application No. 60/272,565, filed March 1, 2001, the disclosure of which is incorporated herein in its entirety by reference.

### **FIELD OF THE INVENTION**

The present invention relates to the fields of virology and immunology and provides immunogenic HIV peptides for use as reagents for detecting HIV in a biological sample and for use as vaccines for the treatment or prophylaxis of HIV infection.

### **BACKGROUND OF THE INVENTION**

One of the most ravaging diseases of the late twentieth century has been acquired immunodeficiency syndrome (AIDS), caused by infection with the human immunodeficiency virus (HIV). It is estimated that AIDS will have claimed the lives of tens of millions by the year 2000. No HIV vaccine currently exists, and treatment protocols have exhibited limited success due to the high mutation rate of the virus and the rapid establishment of HIV variants. These variants produce proteins that are not recognized and therefore not destroyed by the patient's immune system. Eventually, the immune response of the HIV infected individual becomes highly compromised, and the patient succumbs to opportunistic infection.

Scientists have been searching for many years for B- and T-cell antigenic determinants, or epitopes, of HIV proteins that are immunogenic and therefore capable of inducing a protective or therapeutic immune response to either protect against risk of infection by HIV or to reduce an existing HIV infection.

Many researchers have concentrated on the envelope protein (env) of HIV as a source of potential immunogenic epitopes because it is present on the surface of the virus and is therefore a primary target of the immune system. The first experimental vaccines against HIV evaluated in clinical trials were designed to present envelope proteins or peptides to the immune system. However, neutralizing immune responses against these peptides failed to recognize diverse isolates of HIV and individuals were not protected from infection.

Researchers have also considered the use of a live, attenuated HIV strain as a vaccine. Several individuals in Australia have been identified who are infected with HIV, but appear to be free from disease. Virus isolated from these patients has been sequenced and was found to have deletions in the nef gene and a portion of the long terminal repeat (LTR). It is believed that administration of this attenuated virus or a synthetic form of this virus might confer some protection against infection. However, concern that the virus might revert to a pathological strain after administration to a healthy individual has greatly inhibited research in this area. These concerns have been confirmed by experiments in which monkeys inoculated with attenuated simian immunodeficiency virus (SIV) strains began to exhibit clinical symptoms of AIDS two years after vaccination, indicating the potential for pathogenic reversion of the attenuated virus.

Therefore, a long-felt and desperate need exists for the identification of immunogenic HIV peptides that can be used in a clinical setting as an HIV vaccine through their ability to induce a T- or B- cell response and thereby to protect against HIV infection. In addition, there is a need for immunogenic HIV peptides in both clinical and laboratory settings to study HIV infection and how they affect the host.

#### SUMMARY OF THE INVENTION

Immunogenic HIV peptides and methods of use are provided. Each HIV peptide includes epitopes that are immunoreactive with cytotoxic T lymphocytes (CTLs) from HIV-positive individuals and binds to antibodies that are immunoreactive with the assembled class I major histocompatibility complex (MHC) structure. The preferred HIV genome is the HIV-1 E subtype. The HIV-

positive individuals from whom the CTLs are collected are preferably Asian, particularly those from Thailand.

Preferably, the peptide is an isolated, recombinant or synthetic peptide or epitope containing between nine and eleven amino acid residues within certain specified regions of the HIV genome according to the numbering in the HIV strain HXB2 as set forth herein.

Most preferably, the peptide is a peptide or epitope containing between nine and eleven amino acid residues within the following regions of the HIV genome according to the numbering in HXB2:

pol 248-257, 272-281, 571-579

env gp120 6-15, 309-318, 340-348

env gp41 762-770

Alternatively, the HIV peptide has one or more of the amino acid sequences set forth in the attached Sequence Listing and conservative variations thereof or binds to an antibody specifically immunoreactive with one or more peptides having the amino acid sequences set forth in the Sequence Listing and conservative variations thereof. In a most preferred embodiment, the HIV peptide has one or more of the amino acid sequences set forth in SEQ ID NOS: 1-7, and conservative variations thereof or binds to an antibody specifically immunoreactive with one or more peptides having the amino acid sequences of SEQ ID NOS: 1-7, and conservative variations thereof.

Each peptide is useful, alone or combination with other peptides described herein, as a reagent for studying the pathogenesis of HIV. In particular, the reagent can be used to identify the types of immune responses that confer protection against HIV infection or reduce progression of the disease to AIDS. In addition, the peptide is useful as a reagent for monitoring drug efficacy in clinical trials or treatment regimens in patients who are undergoing HIV therapy.

One or more of the peptides are also useful as a vaccine composition when combined with a pharmaceutical carrier for the prophylaxis, treatment or prevention of HIV infection. The vaccine composition is administered to an individual prior to HIV exposure to minimize or prevent HIV infection or is administered after a patient has been infected to reduce the severity of infection and

retard or halt progression to AIDS or reverse the course of the disease after an AIDS diagnosis.

It is therefore an object of the present invention to provide an immunogenic peptide that reacts with antibodies, helper T lymphocytes or cytotoxic T lymphocytes from HIV-positive patients, particularly those that have demonstrated a resistance to AIDS.

It is a further object of the present invention to provide a vaccine for the prevention or treatment of HIV infection.

It is a further object of the present invention to provide an HIV vaccine that confers protection against a wide variety of HIV strains and variants.

It is a further object of the present invention to provide a safe HIV vaccine that cannot revert to a pathogenic state.

It is a further object of the present invention to provide a research tool or reagent to study HIV pathogenesis.

It is a further object of the present invention to provide a research tool or reagent to monitor drug efficacy in clinical trials or treatment regimens.

Other features, objects and advantages of the invention and its preferred embodiments will become apparent from the detailed description that follows.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A, 1B, 1C, 1D, and 1E are bar graphs showing percent specific lysis of some of the peptides described herein. Figures 1A, 1B and 1C provide bar graphs showing the percent specific lysis or CTL recognition of HIV peptides in samples taken from HLA-A11 positive HIV-infected female sex workers as shown in Table 2A at E:T ratios of 30:1 (solid bars) and 10:1 (shaded bars) except for donors 140 (10:1), 3:1), 426 (60:1, 50:1) and 163 (60:1, 30:1). Figures 1D and 1E provide bar graphs showing the percent specific lysis or CTL recognition of HIV peptides in samples taken from HLA-A11 negative HIV-infected female sex workers as shown in Table 2B. Each subject's HLA type is listed beside the subject number. Subject 473 was tested against pools of HLA-A2 and -A11 peptides. CTL assays were performed at E:T ratios of 60:1 (solid bars)

and 30:1 (shaded bars). Subjects 158 and 473 were only tested at an E:T ratio of 50:1. For Figures 1A, 1B, 1C, 1D, and 1E, peripheral blood monocytes cultures (PBMCs) were stimulated *in vitro* with pools of peptide and after 16 to 20 days in culture were tested against target cells labeled with individual peptides. Subject identification numbers are listed at the top of each graph. The cut-off for a positive CTL response (antigen specific lysis  $\geq 10\%$ ) is indicated by a dotted line. The HIV protein origin of the peptide is abbreviated as follows: G=gag, P=pol, E=env, and N=nef.

#### DETAILED DESCRIPTION OF THE INVENTION

Immunogenic HIV peptides and methods of use are provided. Each HIV peptide contains epitopes that are immunoreactive with cytotoxic T lymphocytes (CTLs) from HIV-positive individuals. Therefore, the peptides bind with specificity to the T-cell receptors of CTLs. In addition, each peptide binds to antibodies that are immunoreactive with the assembled class I major histocompatibility complex (MHC) structure, preferably the human leukocyte antigen (HLA) locus HLA-A11 structure, which has processed and is presenting antigen.

Preferably, the peptide is an isolated, recombinant, or synthetic peptide or epitope containing between nine and eleven amino acid residues within the following regions of the HIV genome according to the numbering in HXB2 (a subtype B isolate described by Korber *et al.*, Numbering Positions in HIV Relative to HXB2CG. In HIV MOLECULAR IMMUNOLOGY DATABASE. B. Korber, J. Moore, C. Brandler, R. Koup, B. Haynes and B. Walker, eds. Los Alamos National Laboratories, Theoretical Biology and Biophysics, Los Alamos, New Mexico, p. IV-27, 1998) and may be isolated from a mixture of fragmented proteins, produced recombinantly, or produced synthetically:

pol 248-257, 272-281, 571-579

env gp120 6-15, 309-318, 340-348

env gp41 762-770

gag p17 83-91, 118-127

gag p15 376-384

/  
pol 157-166, 894-903, 918-926  
vif 84-93  
tat 20-29  
rev 107-115  
env gp120 109-117, 244-252, 371-379, 433-442,  
env gp41 675-683  
gag p17 19-28, 106-115  
gag p24 221-229, 254-263, 256-264, 282-290, 352-361  
gag p15 444-452  
pol 158-166, 160-168, 169-177, 212-221, 229-238, 238-247, 249-258, 370-378,  
370-379, 496-505, 612-620, 650-659, 751-760, 943-951, 991-999  
vif 132-141, 159-168, 160-168, 160-169  
vpr 4-12, 9-18  
tat 80-89  
rev 6-14  
env gp120 162-171, 194, 202, 194-203, 208-216, 208-217, 359-368, 413-421  
env gp41 528-537, 721-729, 825-833  
nef 96-105, 109-118, 119-128, 147-156, 183-192, 188-197, 196-205

More preferably, the peptide is an isolated, recombinant, or synthetic peptide or epitope containing between nine and eleven amino acid residues within the following regions of the HIV genome according to the numbering in HXB2:

pol 248-257, 272-281, 571-579  
env gp120 6-15, 309-318, 340-348  
env gp41 762-770  
gag p17 83-91, 118-127  
gag p15 376-384  
pol 157-166, 894-903, 918-926  
vif 84-93  
tat 20-29  
rev 107-115  
env gp120 109-117, 244-252, 371-379, 433-442,  
env gp41 675-683

Most preferably, the peptide is an isolated, recombinant, or synthetic peptide or epitope containing between nine and eleven amino acid residues within the following regions of the HIV genome according to the numbering in HXB2:

pol 248-257, 272-281, 571-579

env gp120 6-15, 309-318, 340-348

env gp41 762-770

Alternatively, the HIV peptide has one or more of the amino acid sequences set forth in SEQ ID NOS:1-69 and conservative variations thereof, or binds to an antibody specifically immunoreactive with one or more peptides having the amino acid sequences of SEQ ID NOS: 1-69 and conservative variations thereof. In a more preferred alternative, the HIV peptide has one or more of the amino acid sequences set forth in SEQ ID NOS: 1-21, and conservative variations thereof or binds to an antibody specifically immunoreactive with one or more peptides having the amino acid sequences of SEQ ID NOS: 1-21, and conservative variations thereof. In a most preferred alternative, the HIV peptide has one or more of the amino acid sequences set forth in SEQ ID NOS: 1-7, and conservative variations thereof or binds to an antibody specifically immunoreactive with one or more peptides having the amino acid sequences of SEQ ID NOS: 1-7, and conservative variations thereof.

The region and sequence number of the HIV genome according to the numbering in HXB2 described above, preferred amino acid sequences, and corresponding SEQ ID NOS assigned herein, are set forth below in Table 1.

The preferred HIV genome is the genome of the HIV-1 E subtype. The preferred HIV-positive individuals from whom the CTLs are collected are Asian patients, particularly those from Thailand. Most preferably, the CTLs are from Asian patients who are HIV-positive, but have exhibited a resistance to the progression of the disease to AIDS. Immunoreactivity with CTLs can be determined using assays and methods well known to those skilled in the art such as cell lysis as determined by detecting the release of label, such as radioactivity, from lysed cells.

Each of the peptides described above are useful, either alone or in combination, as reagents for studying the pathogenesis of HIV. In particular, the



reagents can be used to identify the types of immune responses that confer protection against HIV infection or reduce progression of the disease to AIDS. In particular, the peptides can be used to study the role of cytotoxic T lymphocytes in protecting individuals from developing AIDS, thereby identifying human resistance factors. For example, one or more of the peptides are used in a traditional CTL assay or are used in flow cytometry to identify T cells in blood as taught by Altman, J.D., *et al.*, *Science* 274(5284):94-96(1996), and Ogg, G.S., *et al.*, *Science* 279(5359):2103-2106(1998). Similarities between the peptide-binding T cells of various patients are useful in developing therapies against HIV.

In addition, the peptides are useful as reagents in assays or methods for monitoring drug efficacy in clinical trials or treatment regimens in patients who are undergoing HIV therapy. In accordance with the drug monitoring or treatment evaluation method, a patient sample is combined with a peptide reagent for the detection of antibodies or T-cell receptors that bind with specificity to the reagent. The peptide can be used in solution phase, or can be labeled or immobilized or complexed with other reagents such as a solid phase or MHC molecule to facilitate the detection of peptide-specific antibody or peptide-specific T cells. An increase in the concentration of peptide-reactivity indicates that the patient is mounting an immune response to the virus and may be successfully clearing the virus, or that an HIV vaccine is successfully inducing immunity. A decrease in the concentration could indicate that a drug therapy is being successful. Alternatively, the peptides are useful for the prognosis of disease in an HIV-positive individual who may or may not be receiving drug therapy wherein the detection of or an increase in the concentration of peptide complexes indicates that the individual may be resistant to progression of the disease to AIDS.

Furthermore, one or more of the peptides are useful when combined with a pharmaceutical carrier as a vaccine composition for the prophylaxis or prevention of HIV infection. The vaccine composition is administered to an individual prior to HIV exposure to minimize or prevent HIV infection or is administered to a patient after infection with HIV to reduce the severity of infection and retard or halt progression to AIDS.

### Definitions

The terms "a", "an" and "the" as used herein are defined to mean "one or more" and include the plural unless the context is inappropriate.

By "isolated" is meant peptide free from at least some of the components with which it naturally occurs.

"Peptides," "polypeptides" and "oligopeptides" are used interchangeably and are defined herein as chains of amino acids (typically L-amino acids) in which carbons are linked through peptide bonds formed by a condensation reaction between the carboxyl group of the carbon of one amino acid and the amino group of the carbon of another amino acid. The terminal amino acid at one end of the chain (*i.e.*, the amino terminal) has a free amino group, while the terminal amino acid at the other end of the chain (*i.e.*, the carboxy terminal) has a free carboxyl group. As such, the term "amino terminus" (abbreviated N-terminus) refers to the free amino group on the amino acid at the amino terminal of the peptide, or to the amino group (imino group when participating in a peptide bond) of an amino acid at any other location within the peptide. Similarly, the term "carboxy terminus" (abbreviated C-terminus) refers to the free carboxyl group on the amino acid at the carboxy terminus of a peptide, or to the carboxyl group of an amino acid at any other location within the peptide.

Typically, the amino acids making up a peptide are numbered in order, starting at the amino terminal and increasing in the direction of the carboxy terminal of the peptide. Thus, when one amino acid is said to "follow" another, that amino acid is positioned closer to the carboxy terminal of the peptide than the "preceding" amino acid.

The term "residue" is used herein to refer to an amino acid (D or L) or an amino acid mimetic that is incorporated into a oligopeptide by an amide bond or an amide bond mimetic. As such, the amino acid may be a naturally occurring amino acid or, unless otherwise limited, may encompass known analogs of natural amino acids that function in a manner similar to the naturally occurring amino acids (*i.e.*, amino acid mimetics). Moreover, an amide bond mimetic includes peptide backbone modifications well known to those skilled in the art.

The phrase "consisting essentially of" is used herein to exclude any elements that would substantially alter the essential properties of the immunogenic peptides to which the phrase refers. Thus, the description of a peptide "consisting essentially of . . ." excludes any amino acid substitutions, additions, or deletions that would substantially alter the biological activity of that peptide.

"Antigen" refers to an entity or fragment thereof which can induce an immune response in a mammal. The term includes immunogens and regions responsible for antigenicity or antigenic determinants.

"Antigenic determinant" refers to a region of a protein recognized by an antibody or T cell receptor, *e.g.*, in serum raised against wild-type protein.

The phrases "specifically binds to a peptide" or "specifically immunoreactive with", when referring to an antibody or T cell receptor, refers to a binding reaction which is determinative of the presence of the peptide, or an antibody or T cell receptor to the peptide, in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies or T-cell receptors bind preferentially to a particular peptide and do not bind in a significant amount to other proteins present in the sample. Specific binding to a peptide under such conditions requires an antibody or T cell that is selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. *See*, Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

"Conservatively variations" or "conservative modified variations" of a particular sequence refers to amino acids encoded by nucleic acids, which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given peptide. Such nucleic acid variations are

silent variations, which are one species of conservatively modified variations. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques. Accordingly, each silent variation of a nucleic acid, which encodes a peptide, is implicit in any described amino acid sequence. Furthermore, one of skill will recognize that individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are conservatively modified variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Serine (S), Threonine (T);
- 2) Aspartic acid (D), Glutamic acid (E);
- 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Two polypeptides are said to be "identical" if the sequence of amino acid residues in the two sequences is the same when aligned for maximum correspondence. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (U.S.A.)* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

The term "substantial identity" means that a polypeptide comprises a sequence that has at least 85% sequence identity or homology, preferably 90%, more preferably 95% or more, compared to a reference sequence over a comparison

window of about 10 to about 20 amino acids. Another indication that polypeptide sequences are substantially identical is if one peptide is immunologically reactive with antibodies raised against the disclosed peptide. Thus, the peptides of the invention include peptides immunologically reactive with antibodies raised against the disclosed immunogenic peptides.

#### Synthetic Peptides

The peptides described herein generally contain from about 9 to about 35 amino acid residues, more preferably, from about 9 to about 20 amino acid residues and, even more preferably, from about 9 to about 11 amino acid residues. Because the peptides are relatively short in length, they can be prepared using any of a number of chemical peptide synthesis techniques well known to those of ordinary skill in the art including both solution methods and solid phase methods, with solid phase synthesis being presently preferred.

In particular, solid phase synthesis in which the C-terminal amino acid of the peptide sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence is the preferred method for preparing the peptides. Techniques for solid phase synthesis are described by Merrifield, *et al.*, *J. Am. Chem. Soc.* 85:2149-2156(1963). Many automated systems for performing solid phase peptide synthesis are commercially available.

Solid phase synthesis is started from the carboxy-terminal end (*i.e.*, the C-terminus) of the peptide by coupling a protected amino acid via its carboxyl group to a suitable solid support. The solid support used is not a critical feature of the present invention provided that it is capable of binding to the carboxyl group while remaining substantially inert to the reagents utilized in the peptide synthesis procedure. For example, a starting material can be prepared by attaching an amino-protected amino acid via a benzyl ester linkage to a chloromethylated resin or a hydroxymethyl resin or via an amide bond to a benzhydrylamine (BHA) resin or p-methylbenzhydrylamine (MBHA) resin. Materials suitable for use as solid supports are well known to those of skill in the art and include, but are not limited to, the following: halomethyl resins, such as chloromethyl resin or bromomethyl resin; hydroxymethyl resins; phenol resins, such as 4-(a-[2,4-dimethoxyphenyl]-Fmoc-

aminomethyl)phenoxy resin; tert-alkyloxycarbonyl-hydrazidated resins, and the like. Such resins are commercially available and their methods of preparation are known to those of ordinary skill in the art.

The acid form of the peptides may be prepared by the solid phase peptide synthesis procedure using a benzyl ester resin as a solid support. The corresponding amides may be produced by using benzhydrylamine or methylbenzhydrylamine resin as the solid support. Those skilled in the art will recognize that when the BHA or MBHA resin is used, treatment with anhydrous hydrofluoric acid to cleave the peptide from the solid support produces a peptide having a terminal amide group.

The  $\alpha$ -amino group of each amino acid used in the synthesis should be protected during the coupling reaction to prevent side reactions involving the reactive  $\alpha$ -amino function. Certain amino acids also contain reactive side-chain functional groups (*e.g.*, sulfhydryl, amino, carboxyl, hydroxyl, *etc.*), which must also be protected with appropriate protecting groups to prevent chemical reactions from occurring at those sites during the peptide synthesis. Protecting groups are well known to those of skill in the art. *See, for example, The Peptides: Analysis, Synthesis, Biology, Vol. 3: Protection of Functional Groups in Peptide Synthesis* (Gross and Meienhofer (eds.), Academic Press, N.Y. (1981)).

A properly selected  $\alpha$ -amino protecting group will render the  $\alpha$ -amino function inert during the coupling reaction, will be readily removable after coupling under conditions that will not remove side chain protecting groups, will not alter the structure of the peptide fragment, and will prevent racemization upon activation immediately prior to coupling. Similarly, side-chain protecting groups must be chosen to render the side chain functional group inert during the synthesis, must be stable under the conditions used to remove the  $\alpha$ -amino protecting group, and must be removable after completion of the peptide synthesis under conditions that will not alter the structure of the peptide.

Illustrative examples of protecting groups for an  $\alpha$ -amino group include, but are not limited to, the following: aromatic urethane-type groups such as, for example, fluorenylmethyloxycarbonyl (Fmoc), carbobenzoxy (Cbz), and substituted benzyloxycarbonyls including p-chlorobenzyloxycarbonyl, o-

chlorobenzylloxycarbonyl, 2,4-dichlorobenzylloxycarbonyl, 2,6-dichlorobenzylloxycarbonyl, *etc.*; aliphatic urethane-type groups such as, for example, butyloxycarbonyl (Boc), t-amylloxycarbonyl, isopropylloxycarbonyl, 2-(p-biphenyl)-isopropylloxycarbonyl, allyloxycarbonyl, *etc.*; and cycloalkyl urethane-type groups such as, for example, cyclopentylloxycarbonyl, cyclohexylloxycarbonyl, cycloheptyloxy-carbonyl, adamantylloxycarbonyl (Adoc), *etc.* In a presently preferred embodiment, fluorenylmethyloxycarbonyl (Fmoc) is the  $\alpha$ -amino protecting group used.

For the side chain amino group present in lysine (Lys), any of the protecting groups described above for the protection of the  $\alpha$ -amino group are suitable. Moreover, other suitable protecting groups include, but are not limited to, the following: butyloxycarbonyl (Boc), p-chlorobenzylloxycarbonyl, p-bromobenzylloxycarbonyl, o-chlorobenzylloxycarbonyl, 2,6-dichlorobenzylloxycarbonyl, 2,4-dichlorobenzyl-oxycarbonyl, o-bromobenzylloxycarbonyl, p-nitrobenzylloxycarbonyl, t-butyloxycarbonyl, isopropylloxycarbonyl, t-amylloxycarbonyl, cyclopentylloxycarbonyl, cyclohexylloxycarbonyl, cycloheptyloxycarbonyl, adamantylloxycarbonyl, p-toluenesulfonyl, *etc.* In a presently preferred embodiment, the side chain amino protecting group for Lys is butyloxycarbonyl (Boc).

For protection of the guanidino group of arginine (Arg), examples of suitable protecting groups include, but are not limited to, the following: nitro, tosyl (Tos), carbobenzoxy (Cbz), adamantylloxycarbonyl (Adoc), butyloxycarbonyl (Boc), 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) and 2,2,5,7,8-pentamethylchloroman-6-sulfonyl (PMC). In a presently preferred embodiment, 4-methoxy-2,3,6-trimethyl-benzenesulfonyl and 2,2,5,7,8-pentamethylchloroman-6-sulfonyl are the protecting group used for Arg.

The hydroxyl group on the side chains of serine (Ser), threonine (Thr) or tyrosine (Tyr) can be protected by a C<sub>1</sub>-C<sub>4</sub> alkyl such as, for example, methyl, ethyl and t-butyl, or by a substituted benzyl such as, for example, p-methoxybenzyl, p-nitrobenzyl, p-chlorobenzyl, o-chlorobenzyl and 2,6-dichlorobenzyl. The preferred aliphatic hydroxyl protecting group for Ser, Thr and Tyr is t-butyl.

The carboxyl group of aspartic acid (Asp) may be protected by, for example, esterification using groups such as benzyl, t-butyl, cyclohexyl, cyclopentyl, and the like. For Asp, t-butyl is the presently preferred protecting group.

The basic imidazole ring in histidine (His) may be protected by, for example, t-butoxymethyl (Bom), butyloxycarbonyl (Boc) and fluorenylmethyloxycarbonyl (Fmoc). In a preferred embodiment, t-butoxymethyl (Bom) is the protecting group used.

Coupling of the amino acids may be accomplished by a variety of techniques known to those of skill in the art. Typical approaches involve either the conversion of the amino acid to a derivative that will render the carboxyl group more susceptible to reaction with the free N-terminal amino group of the peptide fragment, or use of a suitable coupling agent such as, for example, N,N'-dicyclohexylcarbodiimide (DCC) or N,N'-diisopropylcarbodiimide (DIPCDI). Frequently, hydroxybenzotriazole (HOBt) is employed as a catalyst in these coupling reactions.

Generally, synthesis of the peptide is commenced by first coupling the C-terminal amino acid, which is protected at the N-amino position by a protecting group such as fluorenylmethyloxycarbonyl (Fmoc), to a solid support. Prior to coupling of Fmoc-Asn, the Fmoc residue has to be removed from the polymer. Fmoc-Asn can, for example, be coupled to the 4-(a-[2,4-dimethoxyphenyl]-Fmoc-amino-methyl)phenoxy resin using N,N'-dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) at about 25°C for about two hours with stirring. Following the coupling of the Fmoc-protected amino acid to the resin support, the  $\alpha$ -amino protecting group is removed using 20% piperidine in DMF at room temperature.

After removal of the  $\alpha$ -amino protecting group, the remaining Fmoc-protected amino acids are coupled stepwise in the desired order. Appropriately protected amino acids are commercially available from a number of suppliers (e.g., Novartis (Switzerland) or Bachem (California)). As an alternative to the stepwise addition of individual amino acids, appropriately protected peptide fragments consisting of more than one amino acid may also be coupled to the



"growing" peptide. Selection of an appropriate coupling reagent, as explained above, is well known to those of skill in the art. It should be noted that because the immunogenic peptides are relative short in length, this latter approach (*i.e.*, the segment condensation method) is not the most efficient method of peptide synthesis.

Each protected amino acid or amino acid sequence is introduced into the solid phase reactor in excess and the coupling is carried out in a medium of dimethylformamide (DMF), methylene chloride ( $\text{CH}_2\text{Cl}_2$ ), or mixtures thereof. If coupling is incomplete, the coupling reaction may be repeated before deprotection of the N-amino group and addition of the next amino acid. Coupling efficiency may be monitored by a number of means well known to those of skill in the art. A preferred method of monitoring coupling efficiency is by the ninhydrin reaction. Peptide synthesis reactions may be performed automatically using a number of commercially available peptide synthesizers such as the Biosearch 9500™ synthesizer, Biosearch, San Raphael, CA).

The peptide can be cleaved and the protecting groups removed by stirring the insoluble carrier or solid support in anhydrous, liquid hydrogen fluoride (HF) in the presence of anisole and dimethylsulfide at about 0C for about 20 to 90 minutes, preferably 60 minutes; by bubbling hydrogen bromide (HBr) continuously through a 1 mg/10 mL suspension of the resin in trifluoroacetic acid (TFA) for 60 to 360 minutes at about room temperature, depending on the protecting groups selected; or by incubating the solid support inside the reaction column used for the solid phase synthesis with 90% trifluoroacetic acid, 5% water and 5% triethylsilane for about 30 to 60 minutes. Other deprotection methods well known to those of skill in the art may also be used.

The peptides can be isolated and purified from the reaction mixture by means of peptide purification well known to those of skill in the art. For example, the peptides may be purified using known chromatographic procedures such as reverse phase HPLC, gel permeation, ion exchange, size exclusion, affinity, partition, or countercurrent distribution.

### Recombinant Peptides

Although the synthetic peptides are preferably prepared or produced using chemical peptide synthesis techniques such as described above, it will be understood by those of ordinary skill in the art that they can also be prepared by other means including, for example, recombinant techniques. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are found in Sambrook *et al.* (1989) *Molecular Cloning - A Laboratory Manual* (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, (Sambrook). Product information from manufacturers of biological reagents and experimental equipment, such as the SIGMA Chemical Company (Saint Louis, MO), also provide information useful in known biological methods.

The peptides described herein are derived from HLA-A11 binding peptides in HIV using the following Thai E subtype HIV-1 strains: CM243 (HIVTN2431 for envelope gp120 and gp41) and 93TH253 for all other proteins (gag, pol, vif, vpr, rev, tat and nef). These sequences are available at the HIV Database at the Los Alamos National Laboratories Web site <http://hiv-web.lanl.gov/>, in GenBank, or other commonly used HIV genetic databases. Provided with the peptide sequences described herein, one of skill will recognize a variety of equivalent nucleic acids that encode the peptide. This is because the genetic code requires that each amino acid residue in a peptide is specified by at least one triplet of nucleotides in a nucleic acid, which encodes the peptide. Due to the degeneracy of the genetic code, many amino acids are equivalently coded by more than one triplet of nucleotides. For instance, the triplets CGU, CGC, CGA, CCG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is to be encoded by a nucleotide triplet, the nucleic acid has any of the triplets, which encode arginine. One of skill is thoroughly familiar with the genetic code and its use. An introduction to the subject is found in, for example, chapter 15 of Watson, *et al.*, *Molecular Biology of the Gene* (Fourth Edition, The Benjamin/Cummings Company, Inc., Menlo Park, California (1987)), and the references cited therein.

Although any nucleotide triplet or codon, which encodes an amino acid, can be used to specify the position of the amino acid in a peptide, certain codons are preferred. It is desirable to select codons for elevated expression of an encoded peptide, for example, when the peptide is purified for use as an immunogenic reagent. Codons are selected by reference to species codon bias tables, which show which codons are most typically used by the organism in which the peptide is to be expressed. The codons used frequently by an organism are translated by the more abundant t-RNAs in the cells of the organism. Because the t-RNAs are abundant, translation of the nucleic acid into a peptide by the cellular translation machinery is facilitated. Codon bias tables are available for most organisms. For an introduction to codon bias tables, *see, e.g., Watson, et al., supra.*

#### Conservative Substitutions

In addition, it will be readily apparent to those of ordinary skill in the art that the peptides described herein and the nucleic acid molecules encoding such immunogenic peptides can be subject to various changes, such as insertions, deletions, and substitutions, either conservative or non-conservative, where such changes might provide for certain advantages in their use, *i.e.,* to increase biological activity.

One of skill will appreciate that many conservative variations of nucleic acid constructs yield a functionally identical construct. For example, due to the degeneracy of the genetic code, silent substitutions (*i.e.,* substitutions of a nucleic acid sequence which do not result in an alteration in an encoded peptide) are an implied feature of *every* nucleic acid sequence, which encodes an amino acid. In addition, one of skill will recognize many ways of generating alterations in a given nucleic acid construct. Such well-known methods include site-directed mutagenesis, PCR amplification using degenerate oligonucleotides, exposure of cells containing the nucleic acid to mutagenic agents or radiation, chemical synthesis of a desired oligonucleotide (*e.g.,* in conjunction with ligation and/or cloning to generate large nucleic acids) and other well-known techniques. *See, Gilman and Smith (1979) Gene 8:81-97, Roberts et al. (1987) Nature 328:731-734 and Sambrook, supra.*

Modifications to nucleic acids are evaluated by routine screening techniques in suitable assays for the desired characteristic. For instance, changes in the immunological character of encoded peptides can be detected by an appropriate immunological assay. Modifications of other properties such as nucleic acid hybridization to a complementary nucleic acid, redox or thermal stability of encoded proteins, hydrophobicity, susceptibility to proteolysis, or the tendency to aggregate are all assayed according to standard techniques.

Similarly, conservative amino acid substitutions, in one or a few amino acids in an amino acid sequence of a protein are substituted with different amino acids with highly similar properties (*see*, the definitions section, *supra*), are also readily identified as being highly similar to a disclosed construct. By conservative substitutions is meant replacing an amino acid residue with another, which is biologically and/or chemically similar, *e.g.*, one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are a feature of the present invention.

#### Immunogenic Conjugates

Immunogenic conjugates containing one or more of the synthetic peptides described above, covalently attached to a carrier protein, are also provided. Suitable carrier proteins include, but are not limited to, the following: thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly(D-lysine:D-glutamic acid), influenza, hepatitis B virus core protein, hepatitis B virus recombinant vaccine, and the like.

When the peptide and carrier protein are relatively short in length (*i.e.*, less than about 50 amino acids), they are preferably synthesized using standard chemical peptide synthesis techniques. When both molecules are relatively short, a chimeric molecule is optionally synthesized as a single contiguous polypeptide. Alternatively, the peptide and the carrier molecule can be synthesized separately and then fused chemically.

Solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the

remaining amino acids in the sequence is a preferred method for the chemical synthesis of the immunogenic conjugates provided herein. Techniques for solid phase synthesis are described above.

Alternatively, the immunogenic conjugates are synthesized using recombinant nucleic acid methodology. Generally, this involves creating a nucleic acid sequence that encodes the peptide-carrier protein immunogenic conjugate, placing the nucleic acid in an expression cassette under the control of a particular promoter, expressing the protein in a host, isolating the expressed protein and, if required, renaturing the protein. Techniques sufficient to guide one of skill through such procedures are found in Sambrook, *supra*.

While the peptide and carrier molecule are often joined directly together, one of skill will appreciate that the molecules may be separated by a spacer molecule (e.g., a peptide) consisting of one or more amino acids. Generally, the spacer will have no specific biological activity other than to join the immunogenic peptide to the carrier protein, or to preserve some minimum distance or other spatial relationship between them. However, the constituent amino acids of the spacer may be selected to influence some property of the molecule such as the folding, net charge, or hydrophobicity.

Once expressed, recombinant immunogenic conjugates can be purified according to standard procedures, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Substantially pure compositions of about 50 to 95% homogeneity are preferred, and 80 to 95% or greater homogeneity are most preferred for use as therapeutic agents.

One of skill in the art will recognize that after chemical synthesis, biological expression or purification, the immunogenic conjugates of the present invention may possess a conformation substantially different than the native conformations of the constituent peptides. In this case, it is often necessary to denature and reduce the polypeptide and then to cause the polypeptide to re-fold into the preferred conformation. Methods of reducing and denaturing proteins and inducing re-folding are well known to those of skill in the art.

### Multiepitope polypeptides

In an alternative embodiment, the immunogenic peptides described herein are combined into multiepitope, or polyepitope, polypeptides or proteins. Typically, 2 to 12 of the immunogenic peptides are fused into a single polypeptide by recombinant or synthetic techniques.

In recombinant procedures, multiepitope proteins are made by ligating synthetic or recombinant nucleic acids, which encode immunogenic peptides. These nucleic acids are ligated enzymatically (*e.g.*, using a DNA ligase enzyme) or synthetically. Alternatively, a single nucleic acid molecule is synthesized which encodes multiple immunogenic peptides. In either case, the resulting nucleic acid encodes multiple immunogenic peptides, all in the same reading frame. Thus, the translated polypeptide contains two or more immunogenic peptide domains.

When the multiepitope polypeptides are produced by automated chemical synthetic procedures, concatamers of peptides are coupled directly. This is performed chemically by joining peptides using standard chemical methods. Alternatively, a polypeptide is synthetically produced that encodes multiple immunogenic peptides.

Chemical or recombinant linker regions are optionally included between immunogenic peptide domains to facilitate presentation of the domains to antibodies, which bind the domains. In preferred embodiments, 10-50 amino acids are inserted between immunogenic domains. Essentially any amino acid, or chemical moiety, which forms amide and carboxyl linkages, can be used as a linker.

### Antibody Production

Antibodies that bind with specificity to the peptides described above are also provided. The antibodies include individual, allelic, strain, or species variants, and fragments thereof, both in their naturally occurring (full-length) forms and in recombinant forms. Additionally, antibodies are raised to these peptides in either their native configurations or in non-native configurations. Anti-idiotypic antibodies can also be generated. Many methods of making antibodies are known to persons of skill. The antibodies are useful as research tools for the isolation of additional quantities of the antigenic peptides and for studying the pathogenesis of

HIV in general. The antibodies may also be useful therapeutically for passive immunization of an HIV-infected patient.

The following discussion is presented as a general overview of the techniques available for the production of antibodies; however, one of skill will recognize that many variations upon the following methods are known.

A number of immunogens are used to produce antibodies specifically reactive with peptides. Recombinant or synthetic peptides of nine amino acids in length, or greater, selected from the peptides disclosed herein are the preferred peptide immunogens for the production of monoclonal or polyclonal antibodies. In one class of preferred embodiments, an immunogenic peptide conjugate is also included as an immunogen. The peptides are used either in pure, partially pure or impure form.

Recombinant peptides are expressed in eukaryotic or prokaryotic cells and purified using standard techniques. The peptide, or a synthetic version thereof, is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies can be generated for subsequent use in immunoassays to measure the presence and quantity of the peptide.

Methods of producing polyclonal antibodies are known to those of skill in the art. In brief, an immunogen, preferably a purified peptide, a peptide coupled to an appropriate carrier (*e.g.*, GST, keyhole limpet hemocyanin, *etc.*), or a peptide incorporated into an immunization vector such as a recombinant vaccinia virus is mixed with an adjuvant and animals are immunized with the mixture. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the peptide of interest. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the peptide is performed where desired.

Antibodies, including binding fragments and single chain recombinant versions thereof, against fragments peptides are raised by immunizing animals, *e.g.*, using immunogenic conjugates comprising a peptide fragment covalently attached (conjugated) to a carrier proteins as described above. Typically, the immunogen of interest is a peptide of at least about 3 amino acids, more

typically the peptide is 5 amino acids in length, preferably, the fragment is 10 amino acids in length and more preferably the fragment is 15 amino acids in length or greater. Often, the fragment is about 20 amino acids in length. The immunogenic conjugates are typically prepared by coupling the peptide to a carrier protein (*e.g.*, as a fusion protein) or, alternatively, they are recombinantly expressed in an immunization vector. Antigenic determinants on peptides to which antibodies bind are typically 3 to 10 amino acids in length.

Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies are screened for binding to normal or modified peptides, or screened for agonistic or antagonistic activity. Specific monoclonal and polyclonal antibodies will usually bind with a  $K_D$  of at least about 0.1 mM, more usually at least about 50 mM, and most preferably at least about 1 mM or better. Often, specific monoclonal antibodies bind with a  $K_D$  of 0.1 mM or better.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, *etc.* Description of techniques for preparing such monoclonal antibodies is found in Kohler and Milstein (1975) *Nature* 256: 495-497. Summarized briefly, this method proceeds by injecting an animal with an immunogen, *i.e.*, an immunogenic peptide of the present invention either alone or optionally linked to a carrier protein. The animal is then sacrificed and cells taken from its spleen, which are fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing *in vitro*. The population of hybridomas is then screened to isolate individual clones, each of which secrete a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic substance.

Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells is enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate (preferably



mammalian) host. The peptides and antibodies of the present invention are used with or without modification, and include chimeric antibodies such as humanized murine antibodies. Other suitable techniques involve selection of libraries of recombinant antibodies in phage or similar vectors. See, Huse *et al.* (1989) *Science* 246: 1275-1281; and Ward, *et al.* (1989) *Nature* 341: 544-546.

Frequently, the peptides and antibodies will be labeled by joining, either covalently or non covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionucleotides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced. See, Cabilly, U.S. Patent No. 4,816,567; and Queen *et al.* *Proc. Nat'l Acad. Sci. USA* 86: 10029-10033(1989).

As mentioned above, the antibodies provided herein can be used in affinity chromatography for isolating additional amounts of the peptides identified herein. Columns are prepared, *e.g.*, with the antibodies linked to a solid support, *e.g.*, particles, such as agarose, Sephadex, or the like, where a cell lysate is passed through the column, washed, and treated with increasing concentrations of a mild denaturant, whereby purified peptides are released. In addition, the antibodies can be used to screen expression libraries for particular expression products, for example, HIV proteins. Usually, the antibodies in such a procedure are labeled with a moiety allowing easy detection of presence of antigen by antibody binding. Moreover, antibodies raised against the immunogenic peptides described herein can also be used to raise anti-idiotypic antibodies. Such antibodies are useful for detecting or diagnosing various pathological or resistance conditions related to the presence of the respective antigens.

#### Immunoassays

Both the peptides described herein and the antibodies that bind with specificity to the peptides are useful as reagents, both as capture agent or labeling agent, in assays to detect a target peptide or antibody. In general, the target

molecule can be quantified by a variety of immunoassay methods. Moreover, the immunoassays can be performed in any of several configurations.

Immunoassays often utilize a labeling agent to specifically bind to and label the binding complex formed by the capture agent and the analyte. The labeling agent may itself be one of the moieties comprising the antibody/analyte complex. Thus, the labeling agent may be a labeled peptide or a labeled anti-peptide antibody. Alternatively, the labeling agent may be a third moiety, such as another antibody, that specifically binds to the antibody/peptide complex, or to a modified capture group (e.g., biotin) which is covalently linked to the peptide or anti-peptide antibody.

Alternatively, the labeling agent can be a streptavidin molecule, which has a fluorescent dye on it and onto which are captured the peptides complexed with MHC (HLA) molecules. These reagents can be used to count single T cells specific for the peptides using commonly used equipment such as flow cytometers, thus providing precise quantitation and phenotype information on the immune response as described by Altman, J.D., *et al.*, *Science* 274(5284):94-96 (1996).

In a preferred embodiment, the labeling agent is an antibody that specifically binds to the capture agent. Such agents are well known to those of skill in the art, and most typically comprise labeled antibodies that specifically bind antibodies of the particular animal species from which the capture agent is derived, such as an anti-idiotypic antibody, or antibodies against a peptide when the peptide is the capture agent. Thus, for example, where the capture agent is a mouse derived anti-peptide antibody, the label agent may be a goat anti-mouse IgG, *i.e.*, an antibody specific to the constant region of the mouse antibody.

Other proteins capable of specifically binding immunoglobulin constant regions, such as streptococcal protein A or protein G are also used as the labeling agent. These proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species.

Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about

five seconds to several hours, preferably from about five minutes to about 24 hours. However, the incubation time will depend upon the assay format, analyte, volume of solution, concentrations, and the like. Usually, the assays are carried out at ambient temperature, although they can be conducted over a range of temperatures, such as 5C to 45C.

#### Non-Competitive Assay Formats

Immunoassays for detecting a peptide or an antibody to a peptide may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of captured analyte (*e.g.*, anti-peptide antibody) is directly measured. In one preferred "sandwich" assay, for example, the capture agent (*e.g.*, immunogenic peptide antibodies) is bound directly to a solid substrate where they are immobilized. These immobilized peptides capture antibodies present in a test sample, such as biological fluid, most preferably blood serum. The antibody thus immobilized is then bound by a labeling agent, such as a second antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived.

Sandwich assays for a peptide or antibody can also be constructed. As described above, the immobilized peptide specifically binds to the antibody present in the sample. A labeled antibody then binds to the already bound antibody. Free-labeled antibody is washed away and the remaining bound labeled antibody is detected (*e.g.*, using a gamma detector where the label is radioactive).

#### Competitive Assay Formats

In competitive assays, the amount of analyte (*e.g.*, immunogenic peptide or antibody to an immunogenic peptide) present in the sample is measured indirectly by measuring the amount of an added (exogenous) analyte displaced (or competed away) from a capture agent (*e.g.*, an antibody or peptide) by the analyte present in the sample. In one competitive assay, a known amount of analyte is added to the sample and the sample is contacted with a capture agent, such as a peptide that specifically binds the analyte. The amount of analyte bound to the peptide is inversely proportional to the concentration of analyte present in the sample.

In a preferred embodiment, the capture agent is immobilized on a solid substrate. The amount of analyte bound to the capture agent is determined either by measuring the amount of antibody present in an antibody/peptide complex or, alternatively, by measuring the amount of remaining uncomplexed antibody. The amount of peptide in a sample to be assayed can also be detected by providing exogenous labeled peptide to the assay.

A hapten inhibition assay is another preferred competitive assay. In this assay, a known analyte, in this case one or more of the peptides described herein, is immobilized on a solid substrate. A known amount of anti-peptide antibody is added to the sample, and the sample is then contacted with the immobilized peptide. In this case, the amount of antibody bound to the immobilized polypeptide is proportional to the amount of peptide present in the sample. Again the amount of immobilized antibody is detected by quantitating either the immobilized fraction of antibody or the fraction of the antibody that remains in solution. Detection may be direct where the antibody is labeled, or indirect where a labeled moiety is subsequently added which specifically binds to the antibody as described above. One of skill will appreciate that the role of the peptide and antibody can be reversed to achieve the same effect for the quantitation of the antibody.

One or more of the peptides described herein or, alternatively, one or more of the antibodies to the peptides is preferably quantified in a biological sample, such as a biological fluid or tissue sample derived from a patient. The detection of the peptides or antibodies indicates that the individual from whom the biological sample was taken is mounting an immune response to the virus. A determination of the quantity of antibodies or protein present in the biological sample provides an indication of the degree of immunity or response to treatment and can therefore be used as a prognostic evaluation.

The sample to be tested or analyzed may be obtained from any biological source and is preferably taken from a human or animal capable of being infected with or harboring the hepatitis A virus. For example, the sample may be a cell sample, tissue sample or biological fluid, such as whole blood, blood serum, blood plasma, urine, semen, saliva, sputum, cerebrospinal fluid, lacrimal fluid,

fermentation fluid, lymph fluid, tissue culture fluid, ascites fluid, synovial fluid, pleural fluid, and the like. The preferred biological sample is a biological fluid from which cells can be removed. The most preferred samples are blood plasma or serum. The biological sample may also be a laboratory research sample such as a cell culture supernatant, viral isolate or viral concentrate. The sample is collected or obtained using methods well known to those skilled in the art.

The sample may be diluted, purified, concentrated, filtered, dissolved, suspended or otherwise manipulated prior to use in the assay. Preferably, a sample containing particulate matter is diluted, filtered, or both diluted and filtered prior to use. The preferred diluent is a buffer solution. Any of a number of standard aqueous buffer solutions, employing one of a variety of buffers, such as phosphate, TRIS detergent, or the like, at physiological pH can be used.

The sample size for the biological fluid sample is preferably between approximately 0.5  $\mu$ l and 1 ml. A preferred biological fluid sample size is between approximately 1 and 100  $\mu$ l. Most preferably, the volume of the biological fluid sample is approximately 10 to 50  $\mu$ l.

After reactivity with one or more of the reagents described herein, the target peptide or antibody in the sample can be detected and quantified by any of a number of means well known to those of skill in the art. These include analytic biochemical methods such as spectrophotometry, radiography, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like, and various immunological methods such as fluid or gel precipitation reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, and the like.

#### Other Assay Formats

Western blot analysis can also be used to detect and quantify the presence of target peptide in the sample. The technique generally includes separating sample products by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the

sample with the antibodies that specifically bind the peptides. The anti-peptide antibodies specifically bind to a peptide fixed on the solid support. These antibodies are directly labeled or, alternatively, they may be subsequently detected using labeled antibodies (*e.g.*, labeled sheep anti-mouse antibodies where the antibody to a peptide is a murine antibody) that specifically bind to the anti-peptide antibody.

Other assay formats include liposome immunoassays (LIAs), which use liposomes designed to bind specific molecules (*e.g.*, antibodies) and release encapsulated reagents or markers. The released chemicals are then detected according to standard techniques.

#### Labels

The labeling agent used to label the peptide or antibody can be, *e.g.*, a peptide, a monoclonal antibody, a polyclonal antibody, an immunogenic peptide or a mosaic polypeptide of immunogenic peptides, or complex such as those described herein, or a polymer such as an affinity matrix, carbohydrate or lipid. Detection may proceed by any known method, such as immunoblotting, western analysis, gel-mobility shift assays, fluorescent *in situ* hybridization analysis (FISH), tracking of radioactive or bioluminescent markers, nuclear magnetic resonance, electron paramagnetic resonance, stopped-flow spectroscopy, column chromatography, capillary electrophoresis, or other methods which track a molecule based upon an alteration in size and/or charge. The particular label or detectable group used in the assay is not a critical aspect of the invention. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well developed in the field of immunoassays and, in general, any label useful in such methods can be applied to the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include magnetic beads (*e.g.* Dynabeads<sup>TM</sup>), fluorescent dyes (*e.g.*, fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (*e.g.*, <sup>3</sup>H, <sup>125</sup>I, <sup>35</sup>S, <sup>14</sup>C, or <sup>32</sup>P), enzymes (*e.g.*, LacZ, CAT, horse radish peroxidase, alkaline phosphatase and others, commonly used as detectable enzymes, either in an EIA or in an ELISA), and

colorimetric labels such as colloidal gold or colored glass or plastic (*e.g.* polystyrene, polypropylene, latex, *etc.*) beads. The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on the sensitivity required, ease of conjugation of the compound, stability requirements, available instrumentation, and disposal provisions.

Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule, such as biotin, is covalently bound to the molecule. The ligand then binds to an anti-ligand, such as streptavidin, molecule, which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-ligands can be used. Where a ligand has a natural anti-ligand, for example, biotin, thyroxine, and cortisol, it can be used in conjunction with the labeled, naturally occurring anti-ligands. Alternatively, any haptenic or antigenic compound can be used in combination with an antibody.

The molecules can also be conjugated directly to signal generating compounds, *e.g.*, by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, *etc.* Chemiluminescent compounds include luciferin, and 2,3-dihydrophthalazinediones, *e.g.*, luminol. For a review of various labeling or signal producing systems, which may be used, see, U.S. Patent No. 4,391,904.

Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence, *e.g.*, by microscopy, visual inspection, via photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels are detected by providing appropriate substrates for the

enzyme and detecting the resulting reaction product. Finally, simple colorimetric labels may be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

#### Solid Phase

As mentioned above, depending upon the assay, various components, including the immunogenic peptide, anti-peptide antibody, or anti-idiotypic antibody, may be bound to a solid surface. Many methods for immobilizing biomolecules to a variety of solid surfaces are known in the art. For instance, the solid surface may be a membrane (*e.g.*, nitrocellulose), a microtiter dish (*e.g.*, PVC, polypropylene, or polystyrene), a test tube (glass or plastic), a dipstick (*e.g.* glass, PVC, polypropylene, polystyrene, latex, and the like), a microcentrifuge tube, or a glass, silica, plastic, metallic or polymer bead. The desired component may be covalently bound, or noncovalently attached through nonspecific bonding.

A wide variety of organic and inorganic polymers, both natural and synthetic may be employed as the material for the solid surface. Illustrative polymers include polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and the like. Other materials, which may be employed, include paper, glass, ceramics, metals, metalloids, semiconductive materials, cements or the like. In addition, substances that form gels, such as proteins (*e.g.*, gelatins), lipopolysaccharides, silicates, agarose and polyacrylamides can be used. Polymers which form several aqueous phases, such as dextrans, polyalkylene glycols or surfactants, such as phospholipids, long chain (12-24 carbon atoms) alkyl



ammonium salts and the like are also suitable. Where the solid surface is porous, various pore sizes may be employed depending upon the nature of the system.

In preparing the surface, a plurality of different materials may be employed, *e.g.*, as laminates, to obtain various properties. For example, protein coatings, such as gelatin can be used to avoid non-specific binding, simplify covalent conjugation, enhance signal detection or the like.

If covalent bonding between a compound and the surface is desired, the surface will usually be polyfunctional or be capable of being polyfunctionalized. Functional groups which may be present on the surface and used for linking can include carboxylic acids, aldehydes, amino groups, cyano groups, ethylenic groups, hydroxyl groups, mercapto groups and the like. The manner of linking a wide variety of compounds to various surfaces is well known and is amply illustrated in the literature.

#### Pharmaceutical Compositions

Vaccine and other pharmaceutical compositions containing one or more of the peptides described herein in a pharmaceutically acceptable carrier are provided. The compositions are useful in therapeutic and prophylactic methods for the treatment, prevention or reduction of HIV infection in humans. Such compositions are suitable for use in a variety of drug delivery systems. Suitable formulations are found in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985). A brief review of methods for drug delivery is provided by Langer, *Science* 249:1 527-1533 (1990).

The compositions are suitable for single administrations or a series of administrations. When given as a series, inoculations subsequent to the initial administration are given to boost the immune response and are typically referred to as booster inoculations.

The pharmaceutical compositions are intended for parenteral, topical, oral or local administration. Preferably, the pharmaceutical compositions are administered parenterally, *e.g.*, intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration that comprise a solution of the agents described above dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of

aqueous carriers may be used, *e.g.*, water, buffered water, 0.4% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient and more preferably at a concentration of 25%-75%.

For aerosol administration, the polypeptides are preferably supplied in finely divided form along with a surfactant and propellant. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. A carrier can also be included, as desired, such as the inclusion of lecithin for intranasal delivery.

The amount administered to the patient will vary depending upon what is being administered, the state of the patient and the manner of administration. In therapeutic applications, compositions are administered to a patient already infected with the HIV virus in an amount sufficient to inhibit spread of the virus, or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as

"therapeutically effective dose." Amounts effective for this use will depend on the severity of the disease, the particular composition, and the weight and general state of the patient. Generally, the dose will be in the range of about 100  $\mu$ g to about 3000  $\mu$ g per day, preferably about 1500  $\mu$ g per day, for a 70 kg patient. Suitable doses for HIV vaccines can be found at <http://camelot.emmes.com/avctn/index.htm>.

More preferably, the peptides are used prophylactically as vaccines. All of the immunogenic peptides disclosed herein can be used as vaccines, either alone, in combination or connected, as in a multiepitope or polypeptide vaccine. The immune response may include the generation of antibodies; activation of cytotoxic T lymphocytes (CTL) against cells presenting the immunogenic peptides, or other mechanisms well known in the art. The preferred dose will be in the range of 100  $\mu$ g to about 3000  $\mu$ g per day, preferably about 1500  $\mu$ g per day, administered in one to six doses.

In a preferred embodiment, the immunogenic peptides are covalently attached (conjugated) to a carrier protein as described above. Useful carrier proteins include, but are not limited to, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly(D-lysine:D-glutamic acid), influenza, hepatitis B virus core protein, hepatitis B virus recombinant vaccine. The vaccines can also contain a physiologically tolerable (acceptable) diluent such as water, phosphate buffered saline, or saline, and further typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are materials well known in the art.

#### DNA Vaccines

In addition, DNA or RNA encoding the immunogenic peptides of the present invention may be introduced into patients to obtain an immune response to the immunogenic peptides, which the nucleic acid encodes. *See, Wolff, et al., Science* 247: 1465-1468 (1990) which describes the use of nucleic acids to produce expression of the immunogenic peptides which the nucleic acids encode, the teachings of which are incorporated herein by reference. Vaccines composed of DNA or RNA encoding immunogenic peptides are commonly referred to in the art as DNA vaccines.

Vaccine compositions containing the immunogenic peptides and nucleic acids of the invention are administered to a patient to elicit a protective immune response against the polypeptide. A "protective immune response" is one, which prevents or inhibits the spread of HIV and, thus, at least partially prevent the symptoms of the disease and its complications. An amount sufficient to accomplish this is defined as an "immunogenically effective dose." Amounts effective for this use will depend on the composition, the manner of administration, the weight and general state of health of the patient, and the judgment of the prescribing physician. For peptide compositions, the general range for the initial immunization (that is for therapeutic or prophylactic administration) is from about 100 µg to about 3000 µg per day, preferably about 1500 µg per day, followed by boosting dosages of the peptide pursuant to a boosting regimen over weeks to months depending upon the patient's response and condition, e.g., by measuring levels of HIV in the patient's blood. For nucleic acids, the same range of doses is preferred.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are intended neither to limit nor define the invention in any manner.

#### Example 1

##### *Identification of MHC Class I CTL Synthetic Peptide Epitopes*

Peptides were synthesized and analyzed in a flow cytometric assay to detect binding to the class I MHC molecule. Peptides that tested positive for class I MHC binding were tested for CTL reactivity with lymphocytes in blood samples from thirteen HIV-positive, Thai, female commercial sex workers.

Forty-nine peptides were synthesized by the Biotechnology Core Facility, National Centers for Infectious Diseases (NCID), Centers for Disease Control and Prevention (CDC, Atlanta, GA), on solid phase support (Wang resin) by Fmoc chemistry as taught by Barany, G. and Merifield, R.B., in "The Peptides", vol.1:1-284 (1980), using an ACT Model MPS 396 multiple peptide synthesizer (Advanced ChemTech, Louisville, KY) according to the manufacturer's protocol. The Wang resin was used so that the resulting peptide product had a free carboxyl

at the C-terminus. The quality of each peptide was determined by amino acid analysis (accuracy), MALDI-TOF mass spectroscopy (accuracy), high performance liquid chromatography (purity), and capillary electrophoresis (purity). All peptides had a purity of 85 to 90%.

#### Subjects

Blood samples from thirteen HIV-1 E subtype infected individuals from a cohort of female sex workers in Chiang Rai, Northern Thailand were collected into 5 ml EDTA tubes or 8 ml Vacutainer CPT™ tubes containing sodium citrate (Becton Dickinson, San Jose, CA). CPT tubes were centrifuged within two hours of collection. All blood was transported overnight at 15-25C from Chiang Rai to the CDC laboratories in Nonthaburi, near Bangkok, Thailand. All subjects used for CTL assays were HIV-1 subtype-E infected as determined using an HIV-1/HIV-2 EIA™ screening kit (Genetic Systems, Redmond, WA) and the Novapath HIV-1 Immunoblot™ Western blot confirmation test (BioRad, Hercules, CA). HIV-1 subtyping was performed using peptide serology as described by Wasi, C. et al., *AIDS* 9:851-857, 1995 and Pau, C-P. et al., *AIDS* 7:331-340, 1993.

Peripheral blood mononuclear cells (PBMCs) were isolated from CPT tubes according to the manufacturer's instructions and cryopreserved at 5 to 10 x 10<sup>6</sup> cells/vial in freezing medium (50% heat inactivated Fetal Bovine Serum (FBS, Hyclone, Logan, UT), 40% RPMI 1640™ (Gibco BRL, Grand Island, NY), 10% dimethylsulfoxide (DMSO, Sigma Chemical Company, St. Louis, MO). Cells were placed overnight at -70C and then stored in liquid nitrogen vapor phase. Specimens were subsequently shipped to CDC, Atlanta, GA, on dry ice or in a liquid nitrogen vapor shipper and stored in liquid nitrogen vapor until use.

B lymphoblastoid cell lines (BLCL) were generated for each subject by transforming with Epstein-Barr Virus (EBV) containing cell culture supernatants as described by Nilsson, K. and Klein, G. *Adv. Cancer Res.* 37:319-380, 1982. EBV containing cell culture supernatants were generated from the chronically EBV-infected marmoset line B95-8 cells (ATCC CRL 1612). For transformation PBMCs (5 to 10 x 10<sup>6</sup> cells) were thawed, washed and incubated with 1 ml of freshly harvested EBV supernatant and 4 ml of medium containing RPMI 1640 supplemented with 20% FBS, 2 µg/ml cyclosporin (Sandoz, East Hanover, NJ), 2%

L-Glutamine (Gibco BRL) and 1% Penicillin-Streptomycin (Gibco BRL). Cells were cultured in 25 cm<sup>2</sup> culture flasks (Corning Costar Corporation, Corning, NY) and fed once to twice weekly. Clusters of transformed cells were normally observed after 14-28 days. Cell cultures were expanded to generate stocks, which were stored frozen.

#### Antibody Assay

The peptides were tested for the ability to bind to two antibodies (A11.1M and AUF5.13), which bind to the assembled complexes of the human MHC molecule, HLA-A11. A11.1M-containing supernatants were generated using hybridoma line HB-164 from the American Type Culture Collection (ATCC, Rockville, MD). This line was maintained in culture medium (RPMI 1640 supplemented with 10% FBS, 2% L-Glutamine and 1% Penicillin-Streptomycin). AUF5.13 also binds assembled HLA-A11 complexes, but has a different epitope specificity. The AUF5.13 was provided by M. Masucci, (Karolinska Institute, Stockholm, Sweden) and was maintained as described by Colombani, J., *et al.*, *Tissue Antigens* 20:161-171(1982). Supernatants from both hybridoma lines were harvested after four to seven days in culture, filter sterilized using a 0.22 µm filter unit (Corning Costar Corporation) and used fresh (or stored at 4C for up to one year) in binding assays.

The HLA-A11 stabilization assay taught by Levitsky et al., *J. Exp. Med.*, 183:915-926 (1996), was employed using T2-A11, an HLA-A11-transfected subline of the peptide transporter mutant cell line T2. The T2-A11 cell line was provided by M. Masucci and was maintained in culture medium supplemented with 200 µg/ml hygromycin B (Sigma Chemical Company) as described by Gavioli, R., *Human Immunology* 49:1-12, 1996). T2-A11 cells (1 x 10<sup>6</sup>) were transferred to 3 ml serum-free Aim V™ medium (Gibco BRL), and 30 µl of candidate A11 binding peptide was added to these cells to a final concentration of 100 µM and incubated for 18 hours at 25C in 25 cm<sup>2</sup> culture flasks. Cells incubated similarly with no peptide served as a negative control. Cells incubated with three EBV EBNA 4 peptides (IVTDFSVIK – SEQ ID NO:131; ISTDFSVIK – SEQ ID NO:132; and IKTDFSVIK – SEQ ID NO:133) served as positive controls to optimize the HLA-

A11 assembly assay when appropriate. Cultures were then transferred to a 37C incubator for two hours.

Cells were stained indirectly, first with 170  $\mu$ l of undiluted culture supernatant from either AUF5.13 or A11.1M followed by 40  $\mu$ l fluorescent isothiocyanate (FITC) conjugated goat anti-mouse IgG (Caltag Laboratories, Bulingame, CA), diluted 1:25 in wash buffer (phosphate buffered saline (PBS) 0.01M pH 7.2, 2% FBS and 0.1% sodium azide). Antibody incubations were 30 minutes each and cells were washed twice between incubations with 2 ml wash buffer and kept on ice or at 4C during staining and washing procedures. Mean fluorescent intensity (MFI) was measured by flow cytometry using a fluorescence activated cell analyzer (FASCan, Becton Dickinson, San Jose, CA) and Cell Quest™ software, version 3.1 (Becton Dickinson). Binding ratios were calculated for each peptide by dividing the MFI of cells incubated with peptide by the MFI of cells incubated with non peptide. Peptides were considered positive for HLA-A11 binding if an MFI ratio of  $\geq 2$  was observed with either antibody in two independent binding experiments. The EBV peptide IVTDFSVIK (SEQ ID NO:15) typically gave an MFI ratio of 3.0 for A11.1M and 8.0 for AUF5.13.

Twenty-six of the synthetic peptides bound to A11 in the flow cytometry assay. These peptides were then tested for reactivity in CTL assays as follows. Two additional peptides were synthesized, but were not tested.

#### CTL Assay

PBMCs were thawed and washed twice in buffered saline (PBS 0.01M, pH 7.2, 2% newborn calf serum, 0.1% glucose) then resuspended in 200  $\mu$ l of CTL medium (RPMI 1640 supplemented with 10% Human AB serum, [Sigma Chemical Company], 330 units/ml recombinant human IL-7 [Genzyme, Cambridge, MA], 10 mM HEPES [Gibco BRL], 2% L-glutamine and 1% Penicillin-Streptomycin) to a concentration of 2 to 4 x 10<sup>7</sup> viable cells/ml in 12 x 75 mm polypropylene tubes (Becton Dickinson). Candidate peptides were then added to cell suspensions as pools of 2 to 6 different peptides to a final concentration of 100-500  $\mu$ M, and cells were placed in a 37C, 5% CO<sub>2</sub> incubator for one to two hours, with occasional agitation. Typically, (A11 and A2/24) pol and env peptides formed individual pools, gag and nef peptides formed another pool and vif, tat and

rev formed another pool. Cell suspensions were then diluted 10-fold to a concentration of  $2$  to  $4 \times 10^6$  cells/ml with culture medium, placed in 2 ml volumes in 24-well flat bottom microtiter plates (Corning Costar Corporation) and incubated for 72 hours at 37C, 5% CO<sub>2</sub>. Delectinized human T-cell growth factor (TCGF, Advanced Biotechnologies Inc., Columbia, MD) and recombinant human IL-2 (Genzyme) were then added at concentrations of 10% and 50 units/ml, respectively. Cultures were split and fed with culture medium supplemented with HEPES, IL-2, and TCGF, in concentrations described above, as needed.

After CTL assays were run, the remaining PBMCs were frozen or restimulated by the addition of an equal number of irradiated (10,000 rads) BLCLs pulsed with appropriate peptides and five to seven fold this number of irradiated (3000 rads) allogeneic normal PBMCs, both in CTL medium. IL-2 and TCGF were added after 72 hours of incubation, and cultures were split and fed as described above.

After 16-21 days of culture with peptide, PBMCs (effectors) were counted, adjusted to generate effector (E) to target (T) ratios of between 100 and 3 to 1 and plated in triplicate in 100  $\mu$ l volumes in 96-well V-bottom plates (Corning Costar Corporation). Healthy BLCLs in the log phase of growth were used as target cells. BLCLs ( $2 \times 10^6$  cells) were washed and resuspended in culture medium at a concentration of  $2 \times 10^6$  cells/ml and labeled with 75-100  $\mu$ Ci of <sup>51</sup>Cr (40 mCi/ml, American Radiolabeled Chemicals Inc., St. Louis, MO), and incubated behind lead shielding at 37C, 5% CO<sub>2</sub> for one hour with occasional agitation. Labeled cells were washed twice with 10 ml culture medium, and  $0.05 \times 10^6$  cells were resuspended in 0.1 ml of culture medium with or without 10  $\mu$ M individual peptides or peptide pools and incubated at 37C, 5% CO<sub>2</sub> with occasional agitation for one hour. Cells were then washed once with 10 ml of culture medium, resuspended, counted, adjusted to 20,000-30,000 cells/ml in culture medium, and 100  $\mu$ l was added in triplicate to effector cells. Cells from each peptide condition, or non-peptide pulsed cells were also incubated alone in 200  $\mu$ l of medium in triplicate without effector cells to generate spontaneous <sup>51</sup>Cr release. Maximum release was similarly determined for each condition by plating 100  $\mu$ l of target cells in triplicate with 100  $\mu$ l of 5% Triton X-100™ in RPMI. Non-specific lysis was



determined for each culture of peptide stimulated PBMCs by incubating at the appropriate E:T ratios with non-peptide pulsed targets. Sealed plates were centrifuged at 1,100 rpm for three minutes, seals were removed and plates were incubated for five hours at 37C, 5% CO<sub>2</sub>. Supernatants were harvested and counted in either a Minaxi Auto-Gamma 5000™ Series Gamma Counter (Packard Instrument Co., Downer Grove, IL) or 1450 MicroBeta Plus™, Liquid Scintillation counter (Wallac, Turku, Finland). For the Packard, 100 µl of supernatant was counted; for liquid scintillation, 50 µl of supernatant was mixed with 190 µl of OptiPhase Supermix™ scintillation cocktail (Wallac). Standard deviations of triplicate wells were calculated using Excel (version 7a) for Windows 95 (Microsoft Corporation, Seattle, OR) and were always < 10%.

Percent specific lysis was calculated using the following formula: % lysis = [experimental release-spontaneous release]/[maximum release-spontaneous release] x 100.

Typically, the spontaneous release was less than 20% of the maximum release. Antigen specific lysis was calculated by subtracting the % lysis of non-peptide-pulsed targets for each expanded culture from the % lysis observed with peptide-pulsed targets. A positive CTL response was defined as antigen specific lysis  $\geq$  10% at one or more E:T ratios unless this degree of lysis was observed only at the lowest E:T ratio, in which case the response was considered negative.

#### Results

The results of the antibody assay and CTL assay are set forth in Table 2A, below. Twenty-six peptides bound HLA-A11.

For the four HLA-A2 subjects (HLA-A11 negative), nine HIV-1 subtype E versions (CM243 and 93TH253) were selected and tested in CTL assays. Because subject 158 is also HLA-A24 positive, the subtype E version of two HLA-A24 epitopes were included. All but one of these peptides (pol 334-342) had one or more substitutions in the E subtype sequence as shown in Table 2B below.

Thirteen peptides were recognized by one or more subjects (see Table 2A, Table 3, Figures 1A, 1B, 1C). Pol responses dominated (69%) followed

by env (62%), gag (38%), and nef (38%). At least seven of the thirteen peptides recognized are believed to be new HLA-A11 CTL epitopes and were identified in pol or env. Each was recognized by up to three individuals. All nine HLA-A11 positive subjects had positive CTL responses to one or more peptides and the number of peptides recognized per subject ranged from 1 to 8 (Figures 1A, 1B, 1C, Table 3). Most subjects had broad responses to two or more HIV proteins.

Table 3 presents the clinical, HLA-A, immunophenotyping and virus load data of the HIV infected FSWs studied. CD4 counts ranged from 30 to 870/ $\mu$ l and viral loads ranged from 889 to 311,324 copies/ml. Nine individuals (69%) were HLAA-A11 positive, the remaining four (31%) were HLA-A2 positive. One individual was HLA-A24 positive.

The CTL response of each subject is set forth in Figures 1A, 1B, and 1C. All nine HLA-A11 positive subjects had a positive CTL response to one or more peptides. Most subjects had broad responses to numerous peptides throughout the HIV genome.

Of those peptides tested in all A11 positive subjects, thirteen peptides were reactive in one or more assays. Six of the thirteen peptides are conserved in that they have matching consensus sequences in five or more subtypes.

The disclosures of all publications cited in this application are hereby incorporated by reference in their entireties in order to more fully describe the state of the art to which this invention pertains.

Although the present process has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

Table 1: HIV Peptides

Region of HIV	Location in HXB2	Peptide Sequence	Sequence Listing Number
pol	248-257	GIPHPAGLKK	SEQ ID NO:1
pol	272-281	SVPLDESEFRK	SEQ ID NO:2
pol	571-579	FVNTPLPVK	SEQ ID NO:3
env gp120	6-15	TQMNWPNLWK	SEQ ID NO:4
env gp120	309-318	ITVGPGQVFY	SEQ ID NO:5
env gp120	340-348	RVLKQVTEK	SEQ ID NO:6
env gp41	762-770	SLCLFSYHR	SEQ ID NO:7
gag p17	83-91	ATLWCVHQR	SEQ ID NO:8
gag p17	118-127	AAADTGSSSK	SEQ ID NO:9
gag p15	376-384	IMMQRGNFK	SEQ ID NO:10
pol	157-166	ISPIDTVPVK	SEQ ID NO:11
pol	894-903	AVFIHNFKRK	SEQ ID NO:12
pol	918-926	IIATDIQTK	SEQ ID NO:13
vif	84-93	GVSIEWRQRK	SEQ ID NO:14
tat	20-29	TACSKCYCKK	SEQ ID NO:15
rev	107-115	SVILGPGTK	SEQ ID NO:16
env gp120	109-117	ISLWDQSLK	SEQ ID NO:17
env gp120	244-252	SVQCTHGIK	SEQ ID NO:18
env gp120	371-379	ITMHHFNCR	SEQ ID NO:19
env gp120	433-442	AMYAPPISGK	SEQ ID NO:20
env gp41	675-683	ITNWLWYIK	SEQ ID NO:21
gag p17	19-28	IRLRPGGKKK	SEQ ID NO:22
gag p17	106-115	EVQKKSQQKK	SEQ ID NO:23
gag p24	221-229	GPIPPGQMR	SEQ ID NO:24
gag p24	254-263	PPIPVGDIYK	SEQ ID NO:25
gag p24	256-264	IPVGDIYKR	SEQ ID NO:26
gag p24	282-290	ILDIRQGPK	SEQ ID NO:27
gag p24	352-361	GVGGPSHKAR	SEQ ID NO:28

Region of HIV	Location in HXB2	Peptide Sequence	Sequence Listing Number
gag p15	444-452	RPGNFPQSK	SEQ ID NO:29
pol	158-166	SPIDTVPVK	SEQ ID NO:30
pol	160-168	IDTVPVKLK	SEQ ID NO:31
pol	169-177	PGMDGPKVK	SEQ ID NO:32
pol	212-221	NTPVFAIKKK	SEQ ID NO:33
pol	229-238	LVDFRELNKR	SEQ ID NO:34
pol	238-247	RTQDFWEVQL	SEQ ID NO:35
pol	249-258	IPHPAGLKKK	SEQ ID NO:36
pol	370-378	TTPDKKHQK	SEQ ID NO:37
pol	370-379	TTPDKKHQKE	SEQ ID NO:38
pol	496-505	IYQEPFKNLK	SEQ ID NO:39
pol	612-620	YVTDRGRQK	SEQ ID NO:40
pol	650-659	IVTDSQYALG	SEQ ID NO:41
pol	751-760	IVANCDKCQL	SEQ ID NO:42
pol	943-951	RDSRDPIWK	SEQ ID NO:43
pol	991-999	AGDDCVAGR	SEQ ID NO:44
vif	132-141	RCEYPSGHNK	SEQ ID NO:45
vif	159-168	IRPPLPSVKK	SEQ ID NO:46
vif	160-168	RPPLPSVKK	SEQ ID NO:47
vif	160-169	RPPLPSVKKL	SEQ ID NO:48
vpr	4-12	APEDQGSQR	SEQ ID NO:49
vpr	9-18	GSQREPYNEW	SEQ ID NO:50
tat	80-89	NPTDPKESKK	SEQ ID NO:51
rev	6-14	GSTDEELLR	SEQ ID NO:52
Env gp120	162-171	TTEL RDKKQK	SEQ ID NO:53
Env gp120	194-202	INCNTSVIK	SEQ ID NO:54
Env gp120	194-203	INCNTSVIKQ	SEQ ID NO:55
Env gp120	208-216	ISFDPIPIH	SEQ ID NO:56
Env gp120	208-217	ISFDPIPIHY	SEQ ID NO:57

Region of HIV	Location in HXB2	Peptide Sequence	Sequence Listing Number
Env gp120	359-368	IIFQPPSGGD	SEQ ID NO:58
Env gp120	413-421	TTTLPCIK	SEQ ID NO:59
env gp41	528-537	STMGAASITL	SEQ ID NO:60
env gp41	721-729	LQTPTHHQR	SEQ ID NO:61
env gp41	825-833	GWTDKVIEVA	SEQ ID NO:62
nef	96-105	GLEGLVYSKK	SEQ ID NO:63
nef	109-118	ILDLWVYHTQ	SEQ ID NO:64
nef	119-128	GFFPDWHNYT	SEQ ID NO:65
nef	147-156	PVDPREVEED	SEQ ID NO:66
nef	183-192	WKFDSALARR	SEQ ID NO:67
nef	188-197	ALARRHIARE	SEQ ID NO:68
nef	196-205	RELRPETYKD	SEQ ID NO:69
gag p17	82-91	IATLWCVHQR	SEQ ID NO:70
pol	313-321	AIFQSSMTK	SEQ ID NO:71
pol	495-505	QIYQEPFKNLK	SEQ ID NO:72
Env gp120	36-46	VTVYYGVPVWR	SEQ ID NO:73
nef	73-82	QVPLRPMTYK	SEQ ID NO:74
nef	83-92	GAFDLSFFLK	SEQ ID NO:75

Table 2A: HLA-A11 Binding Peptides and CTL Recognition in HIV-1 Subtype E Infected Thai Female Sex Workers

Region of HIV	Sequence Numbering in HXB2	Peptide (SEQ ID NO)	Cytometric Assay Binding Ratio		# with CTL Activity/ # tested (%)	# isolates with exact aa seq. match
			A11.1M	AUF5.13		
HIV E subtype HLA-A11 CTL epitopes						
pol	248-257	GIPHPAGLKK (SEQ ID NO:1)	3.2	2.5	2/9 (22)	468
pol	272-281	SVPLDESFRK (SEQ ID NO:2)	5.3	1.1	3/9 (33)	25
pol	571-579	FVNTPLVK (SEQ ID NO:3)	2.9	4.1	1/9 (11)	59
env gp120	6-15	TQMNPWLWK (SEQ ID NO:4)	6.1	6.9	2/9 (22)	17
env gp120	309-318	ITVGPQVFF (SEQ ID NO:5)	2.3	3.9	2/9 (22)	3
env gp120	340-348	RVLKQVTEK (SEQ ID NO:6)	4.6	5.2	2/9 (22)	4
env gp41	762-770	SLCLFSYHR (SEQ ID NO:7)	3.2	7.7	1/9 (11)	142

Region of HIV	Sequence Numbering in HXB2	Peptide (SEQ ID NO)	Cytometric Assay Binding Ratio		# with CTL Activity/ # tested (%)	# isolates with exact aa seq. match
			A11.1M	AUF5.13		
HIV E subtype HLA-A82-9111 CTL epitopes (known in HIV-1 B subtype)						
gag p17	82-91	IATLWCVHQR (SEQ ID NO:70)	4.3	20.3	3/9 (33)	25
pol	313-321	AIFQSSMTK (SEQ ID NO:71)	4.5	9.1	6/9 (67)	217
pol	495-505	QIYQEPFKNLK (SEQ ID NO:72)	3.7	1.0	5/9 (56)	60
env gp120	36-46	VTVYYGVVWR (SEQ ID NO:73)	2.0	4.0	1/9 (11)	35
nef	73-82	QVPLRPMTYK (SEQ ID NO:74)	4.7	1.9	4/9 (44)	498
nef	83-92	GAFDLSFFLK (SEQ ID NO:75)	4.8	1.6	4/9 (44)	29
HLA-A11 binding peptides, no 376-384 CTL activity in this study						
gag p17	118-127	AAADTGSSSK (SEQ ID NO:9)	4.5	7.4	0/6 (0)	15
gag p15	376-384	IMMQRGNFK	5.6	7.1	0/6 (0)	33

Region of HIV	Sequence Numbering in HXB2	Peptide (SEQ ID NO)	Cytometric Assay Binding Ratio		# with CTL Activity/ # tested (%)	# isolates with exact aa seq. match
			A11.1M	AUF5.13		
		(SEQ ID NO:10)				
pol	157-166	ISPDTVPVK (SEQ ID NO:11)	3.5	6.3	0/6 (0)	5
pol	894-903	AVFIHFKRK (SEQ ID NO:12)	2.8	1.1	0/9 (0)	44
pol	918-926	IIATDIQTK (SEQ ID NO:13)	2.5	5.0	0/6 (0)	33
vif	84-93	GVSIEWRQRK (SEQ ID NO:14)	3.1	1.1	0/6 (0)	7
tat	20-29	TACSKCYCKK (SEQ ID NO:15)	2.6	1.0	0/6 (0)	6
rev	107-115	SVILGPGTK (SEQ ID NO:16)	5.3	9.2	0/6 (0)	4
env gp120	109-117	ISLWDQSLK (SEQ ID NO:17)	4.7	7.0	0/9 (0)	391
env gp120	244-252	SVQCETHGIK (SEQ ID NO:18)	3.6	10.3	0/6 (0)	63



Region of HIV	Sequence Numbering in HXB2	Peptide (SEQ ID NO)	Cytometric Assay Binding Ratio		# with CTL Activity/ # tested (%)	# isolates with exact aa seq. match
			A11.1M	AUF5.13		
env gp120	371-379	ITMHHFNCR (SEQ ID NO:19)	2.9	2.3	0/6 (0)	59
env gp120	433-442	AMYAPPISGK (SEQ ID NO:20)	5.2	10.0	0/6 (0)	7
env gp41	675-683	ITNWLWYIK (SEQ ID NO:21)	6.3	8.5	0/6 (0)	87

Table 2B: HLA-A2 and A24 Epitopes and CTL Recognition in HIV-1 Subtype E Infected Thai Female Sex Workers

Region of HIV	Sequence Numbering in HXB2	HLA Restriction	Original subtype B sequence of known CTL epitope	HIV-1 subtype E Sequence Used*	# with CTL Activity/ # tested (%)	# isolates with exact aa seq. match
gag p17	28-36 <sup>A</sup>	A24	KYKLKHIVW (SEQ ID NO:76)	KYMKHLVW (SEQ ID NO:87)	0/1 (0)	14
gag p17	77-85	A2	SLYNTVATL (SEQ ID NO:77)	SLYNTLATL (SEQ ID NO:88)	2/4 (50)	36
Pol	334-342	A2	VITYQYMDL (SEQ ID NO:78)	VITYQYMDL (SEQ ID NO:89)	2/4 (50)	476
Pol	464-472	A2	ILKEPVHGV (SEQ ID NO:79)	ILRIPVHGV (SEQ ID NO:90)	1/4 (25)	3
env gp120	191-200	A2	KLTSNTSV (SEQ ID NO:80)	YRLNCNTSV (SEQ ID NO:91)	1/4 (25)	216
env gp41	586-593 <sup>B</sup>	A24	YIKDQQLL (SEQ ID NO:81)	RYLKDQKLL (SEQ ID NO:92)	0/1 (0)	1
env gp41	747-755	A2	RLVNGSLAL (SEQ ID NO:82)	RLVSGELAL (SEQ ID NO:93)	2/4 (50)	65
env gp41	813-822	A2	SLLNATDIAV (SEQ ID NO:83)	SLLNATAIAV (SEQ ID NO:94)	1/4 (25)	115

Region of HIV	Sequence Numbering in HXB2	HLA Restriction	Original subtype B sequence of known CTL epitope	HIV-1 subtype B Sequence Used*	# with CTL Activity/ # tested (%)	# isolates with exact aa seq. match
env gp41	828-836	A2	RVIEVLQRA (SEQ ID NO:84)	KVIEVAQGA (SEQ ID NO:95)	1/4 (25)	1
Nef	136-145 <sup>c</sup>	A2	PLTFGWCYKL (SEQ ID NO:85)	PLCFGWCFKL (SEQ ID NO:96)	0/4 (0)	57
Nef	180-189 <sup>d</sup>	A2	VLEWRFD SRL (SEQ ID NO:86)	VLIWKFD SAL (SEQ ID NO:97)	0/4 (0)	5

\* Sequence used in CTL assays. Underlined residues indicate a substitution from subtype B to E.

<sup>a</sup> In subtype B HIV-1 this overlaps a known HLA-B8 epitope.

<sup>b</sup> In subtype B HIV-1 this overlaps known HLA-B8, -b14, -B27 and DPw4.2 epitopes

<sup>c</sup> In subtype B HIV-1 this overlaps or is contained within HLA-B7, -B8, -B18, -B35, -B49 and -B57 epitopes.

<sup>d</sup> In subtype B HIV-1 this overlaps larger HLA-A1, -A25, -B8 and -B35 epitopes.

Table 3: Clinical and Immunologic Studies in HIV-1 Subtype E Infected Thais

ID#	HLA-A Locus Type*	Duration of Follow-up (months)	WBC	Lymphocyte (%)	CD4 Number (/MI)	Plasma HIV Load (copies/ml)	CTL Response				
							gag	pol	env	nef	total
053	11	46	6800	29	870	889	1	2	1	2	6
070	11	55	10100	28	283	114,822		4	1		5
140	11	51	8100	23	224	262,255	1	1		2	4
163	11	37	3800	25	180	22,086			1		1
176	11	60	6000	35	530	171,417		1	**	**	1
184	11	57	5600	29	390	1,676		3	1	1	5
197	11	62	11100	18	200	126,215		2	4	2	8
237	11	55	8700	26	520	3,361	1				1
426	11	25	5300	33	280	9,439		4		1	5
125	0203/0207	42	6500	42	490	6,322	1		3		4
144	02/02	53	8400	25	500	97,961	1	2	1		4
158	0207/2407	46	4000	28	30	311,324		1	1		2
473	02/33	28	6400	15	30	67,704					0
		Median	Median	Median	Median	Median	Number (%) of subjects responding to each protein				
		51	6500	28	283	67704	5 (38)	9 (69)	8 (62)	5 (38)	

\* HLA-A11 status was determined by PCR; the A locus type at the other allele was not determined if the subject was HLA-A11 positive.

\*\* Significant CTL responses were seen in subject 176 when *in vitro* stimulated with and tested against pools of env and gag/nef peptides (data not shown).

## WHAT IS CLAIMED IS:

1. An immunogenic HIV peptide comprising one or more epitopes immunoreactive with cytotoxic T lymphocytes from an HIV-positive individual, wherein the peptide binds to antibodies that are immunoreactive with the assembled class I major histocompatibility complex and contains between nine and eleven amino acid residues having an amino acid sequence within the regions of the HIV genome according to the numbering in HXB2 selected from the group consisting of:

pol 248-257, 272-281, 571-579,  
env gp120 6-15, 309-318, 340-348,  
env gp41 762-770,  
gag p17 83-91, 118-127,  
gag p15 376-384,  
pol 157-166, 894-903, 918-926,  
vif 84-93,  
tat 20-29,  
rev 107-115,  
env gp120 109-117, 244-252, 371-379, 433-442,  
env gp41 675-683,  
gag p17 19-28, 106-115,  
gag p24 221-229, 254-263, 256-264, 282-290, 352-361,  
gag p15 444-452,  
pol 158-166, 160-168, 169-177, 212-221, 229-238, 238-247, 249-258, 370-378,  
370-379, 496-505, 612-620, 650-659, 751-760, 943-951, 991-999,  
vif 132-141, 159-168, 160-168, 160-169,  
vpr 4-12, 9-18,  
tat 80-89,  
rev 6-14,  
env gp120 162-171, 194-202, 194-203, 208-216, 208-217, 359-368, 413-421,  
env gp41 528-537, 721-729, 825-833, and  
nef 96-105, 109-118, 119-128, 147-156, 183-192, 188-197, 196-205.

2. The peptide of Claim 1 wherein the class I major histocompatibility complex is human HLA-A11.

3. The peptide of Claim 1 wherein the HIV-positive individual is HIV-1 subtype E positive.

4. The peptide of Claim 1 having an amino acid sequence within the regions of the HIV genome selected from the group consisting of pol 248-257, 272-281, 571-579, env gp120 6-15, 309-318, 340-348, env gp41 762-770, gag p17 83-91, 118-127, gag p15 376-384, pol 157-166, 894-903, 918-926, vif 84-93, tat 20-29, rev 107-115, env gp120 109-117, 244-252, 371-379, 433-442, env gp41 675-683.

5. The peptide of Claim 1 having an amino acid sequence within the regions of the HIV genome selected from the group consisting of pol 248-257, 272-281, 571-579, env gp120 6-15, 309-318, 340-348, env gp41 762-770.

6. The peptide of Claim 1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-69 and conservative variations thereof.

7. The peptide of Claim 1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-21 and conservative variations thereof.

8. The peptide of Claim 1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:1-7 and conservative variations thereof.

9. The peptide of Claim 1 wherein the peptide is recombinant or synthetic.

10. The peptide of Claim 1 wherein the antibodies are A11.1M, AUF5.13 or a combination thereof.

11. A method for monitoring the efficacy of a pharmaceutical anti-HIV drug comprising combining a sample from an HIV-positive patient to whom the drug has been administered with an immunogenic HIV peptide comprising one or more epitopes immunoreactive with cytotoxic T lymphocytes from an HIV-positive individual and detecting the formation of a complex between the peptide and antibodies or T lymphocytes in the sample, wherein the detection of the formation of a complex indicates drug efficacy, wherein the peptide binds to antibodies that are immunoreactive with the assembled class I major histocompatibility complex and contains between nine and eleven amino acid residues having an amino acid sequence within the regions of the HIV genome according to the numbering in HXB2 selected from the group consisting of:  
pol 248-257, 272-281, 571-579,  
env gp120 6-15, 309-318, 340-348,  
env gp41 762-770,  
gag p17 83-91, 118-127,  
gag p15 376-384,  
pol 157-166, 894-903, 918-926,  
vif 84-93,

tat 20-29,  
rev 107-115,  
env gp120 109-117, 244-252, 371-379, 433-442,  
env gp41 675-683,  
gag p17 19-28, 106-115,  
gag p24 221-229, 254-263, 256-264, 282-290, 352-361,  
gag p15 444-452,  
pol 158-166, 160-168, 169-177, 212-221, 229-238, 238-247, 249-258, 370-378,  
370-379, 496-505, 612-620, 650-659, 751-760, 943-951, 991-999,  
vif 132-141, 159-168, 160-168, 160-169,  
vpr 4-12, 9-18,  
tat 80-89,  
rev 6-14,  
env gp120 162-171, 194, 202, 194-203, 208-216, 208-217, 359-368, 413-421,  
env gp41 528-537, 721-729, 825-833, and  
nef 96-105, 109-118, 119-128, 147-156, 183-192, 188-197, 196-205.

12. A prognosis method for determining AIDS resistance comprising combining a sample from an HIV-positive patient with an immunogenic HIV peptide comprising one or more epitopes immunoreactive with cytotoxic T lymphocytes from an HIV-positive individual and detecting the formation of a complex between the peptide and antibodies or T lymphocytes in the sample, wherein the detection of the formation of a complex indicates that the patient is resistant to progression of the disease to AIDS, wherein the peptide binds to antibodies that are immunoreactive with the assembled class I major histocompatibility complex and contains between nine and eleven amino acid residues having an amino acid sequence within the regions of the HIV genome according to the numbering in HXB2 selected from the group consisting of:

pol 248-257, 272-281, 571-579,  
env gp120 6-15, 309-318, 340-348,  
env gp41 762-770,  
gag p17 83-91, 118-127,



gag p15 376-384,  
pol 157-166, 894-903, 918-926,  
vif 84-93,  
tat 20-29,  
rev 107-115,  
env gp120 109-117, 244-252, 371-379, 433-442,  
env gp41 675-683,  
gag p17 19-28, 106-115,  
gag p24 221-229, 254-263, 256-264, 282-290, 352-361,  
gag p15 444-452,  
pol 158-166, 160-168, 169-177, 212-221, 229-238, 238-247, 249-258, 370-378,  
370-379, 496-505, 612-620, 650-659, 751-760, 943-951, 991-999,  
vif 132-141, 159-168, 160-168, 160-169,  
vpr 4-12, 9-18,  
tat 80-89,  
rev 6-14,  
env gp120 162-171, 194, 202, 194-203, 208-216, 208-217, 359-368, 413-421,  
env gp41 528-537, 721-729, 825-833, and  
nef 96-105, 109-118, 119-128, 147-156, 183-192, 188-197, 196-205.

13. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an immunogenic HIV peptide comprising one or more epitopes immunoreactive with cytotoxic T lymphocytes from an HIV-positive individual, wherein the peptide binds to antibodies that are immunoreactive with the assembled class I major histocompatibility complex and contains between nine and eleven amino acid residues having an amino acid sequence within the regions of the HIV genome according to the numbering in HXB2 selected from the group consisting of:

pol 248-257, 272-281, 571-579,  
env gp120 6-15, 309-318, 340-348,  
env gp41 762-770,  
gag p17 83-91, 118-127,

gag p15 376-384,  
pol 157-166, 894-903, 918-926,  
vif 84-93,  
tat 20-29,  
rev 107-115,  
env gp120 109-117, 244-252, 371-379, 433-442,  
env gp41 675-683,  
gag p17 19-28, 106-115,  
gag p24 221-229, 254-263, 256-264, 282-290, 352-361,  
gag p15 444-452,  
pol 158-166, 160-168, 169-177, 212-221, 229-238, 238-247, 249-258, 370-378,  
370-379, 496-505, 612-620, 650-659, 751-760, 943-951, 991-999,  
vif 132-141, 159-168, 160-168, 160-169,  
vpr 4-12, 9-18,  
tat 80-89,  
rev 6-14,  
env gp120 162-171, 194, 202, 194-203, 208-216, 208-217, 359-368, 413-421,  
env gp41 528-537, 721-729, 825-833, and  
nef 96-105, 109-118, 119-128, 147-156, 183-192, 188-197, 196-205.

14. The composition of Claim 13 wherein the composition is a vaccine.

15. The composition of Claim 13 wherein the class I major histocompatibility complex is human HLA-A11.

16. The composition of Claim 13 wherein the HIV-positive individual is HIV-1 subtype E positive.

17. The composition of Claim 13 wherein the peptide has an amino acid sequence selected from the group consisting of SEQ ID NOS:1-69 and conservative variations thereof.

18. A method of inducing an immune response in a human or animal comprising administering to the human or animal an immunologically effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an immunogenic HIV peptide comprising one or more epitopes immunoreactive with cytotoxic T lymphocytes from an HIV-positive individual, wherein the peptide binds to antibodies that are immunoreactive with the assembled class I major histocompatibility complex and contains between nine and eleven amino acid residues having an amino acid sequence within the regions of the HIV genome according to the numbering in HXB2 selected from the group consisting of:

pol 248-257, 272-281, 571-579,  
env gp120 6-15, 309-318, 340-348,  
env gp41 762-770,  
gag p17 83-91, 118-127,  
gag p15 376-384,  
pol 157-166, 894-903, 918-926,  
vif 84-93,  
tat 20-29,  
rev 107-115,  
env gp120 109-117, 244-252, 371-379, 433-442,  
env gp41 675-683,  
gag p17 19-28, 106-115,  
gag p24 221-229, 254-263, 256-264, 282-290, 352-361,  
gag p15 444-452,  
pol 158-166, 160-168, 169-177, 212-221, 229-238, 238-247, 249-258, 370-378,  
370-379, 496-505, 612-620, 650-659, 751-760, 943-951, 991-999,  
vif 132-141, 159-168, 160-168, 160-169,  
vpr 4-12, 9-18,  
tat 80-89,  
rev 6-14,  
env gp120 162-171, 194, 202, 194-203, 208-216, 208-217, 359-368, 413-421,

env gp41 528-537, 721-729, 825-833, and  
nef 96-105, 109-118, 119-128, 147-156, 183-192, 188-197, 196-205.

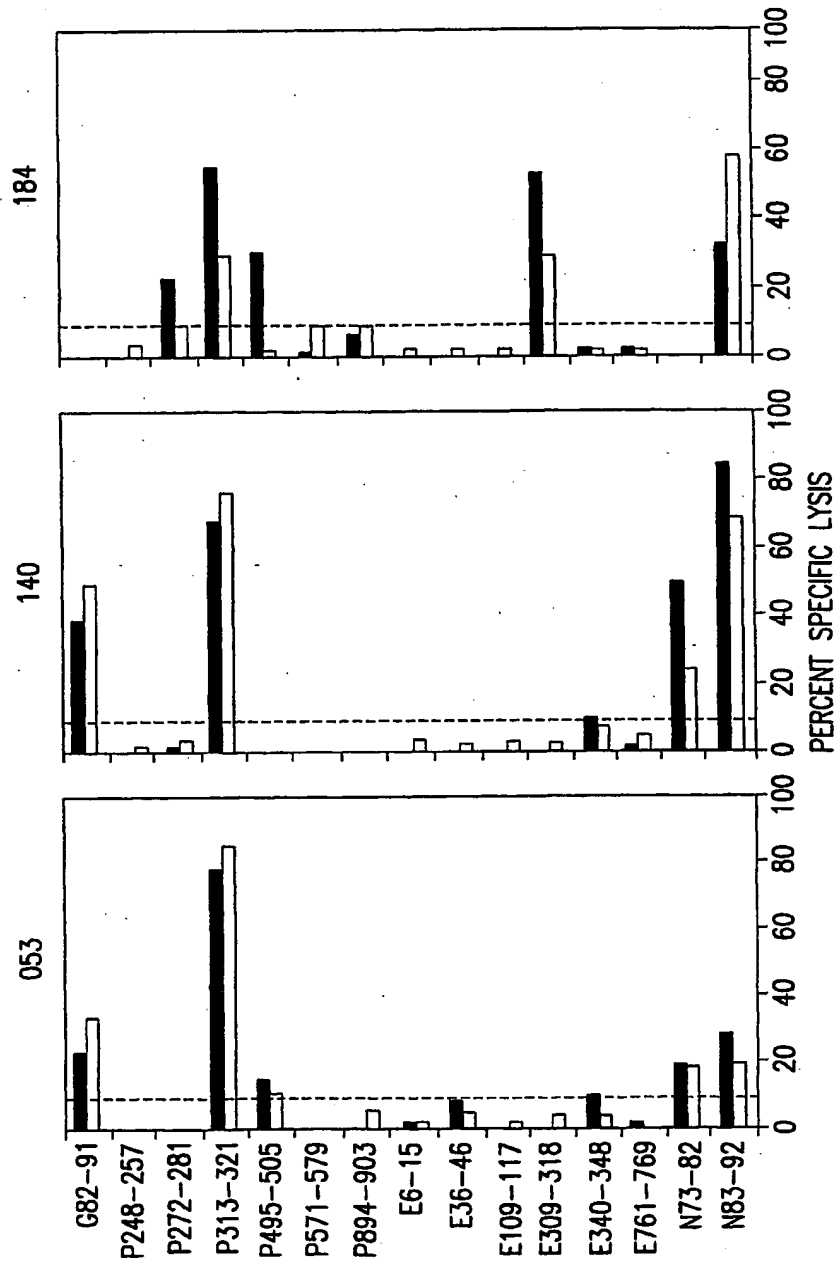
19. The method of Claim 18 wherein the class I major histocompatibility complex is human HLA-A11.

20. The method of Claim 18 wherein the HIV is HIV-1 subtype E.

21. The method of Claim 20 wherein the peptide has an amino acid sequence selected from the group consisting of SEQ ID NOS:1-69 and conservative variations thereof.

22. The method of Claim 20 wherein the antibodies are A11.1M antibodies or AUF5.13 antibodies.

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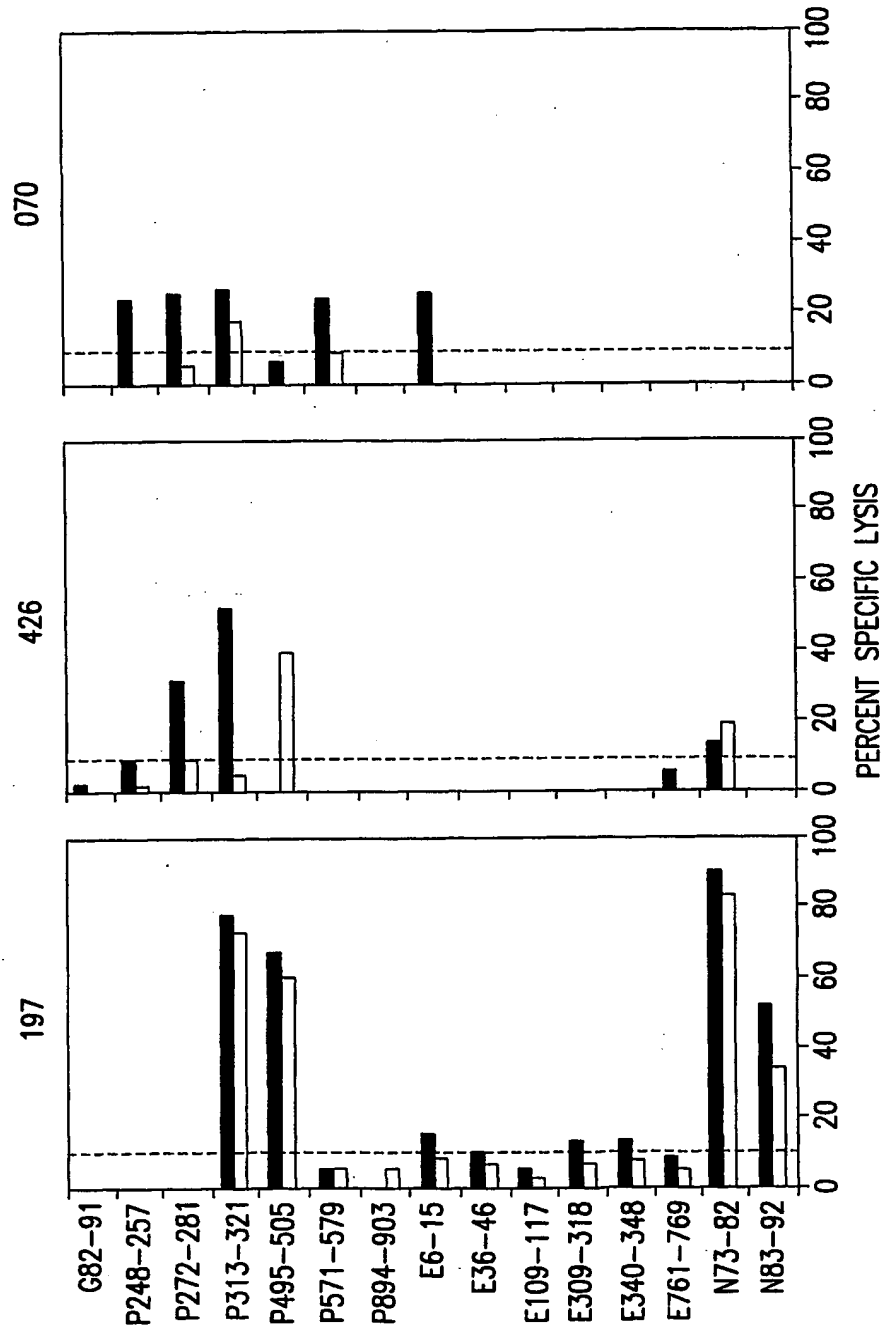


FIG.1B

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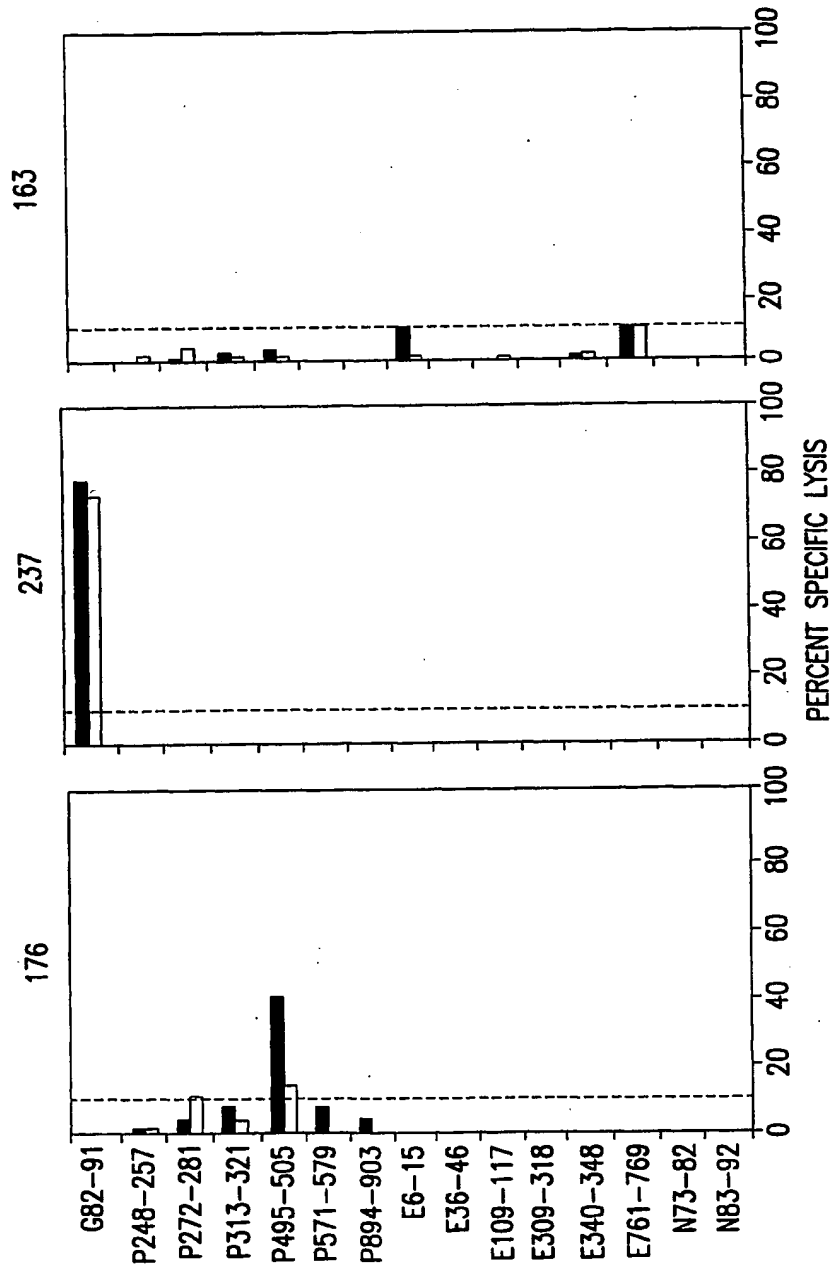


FIG.1C

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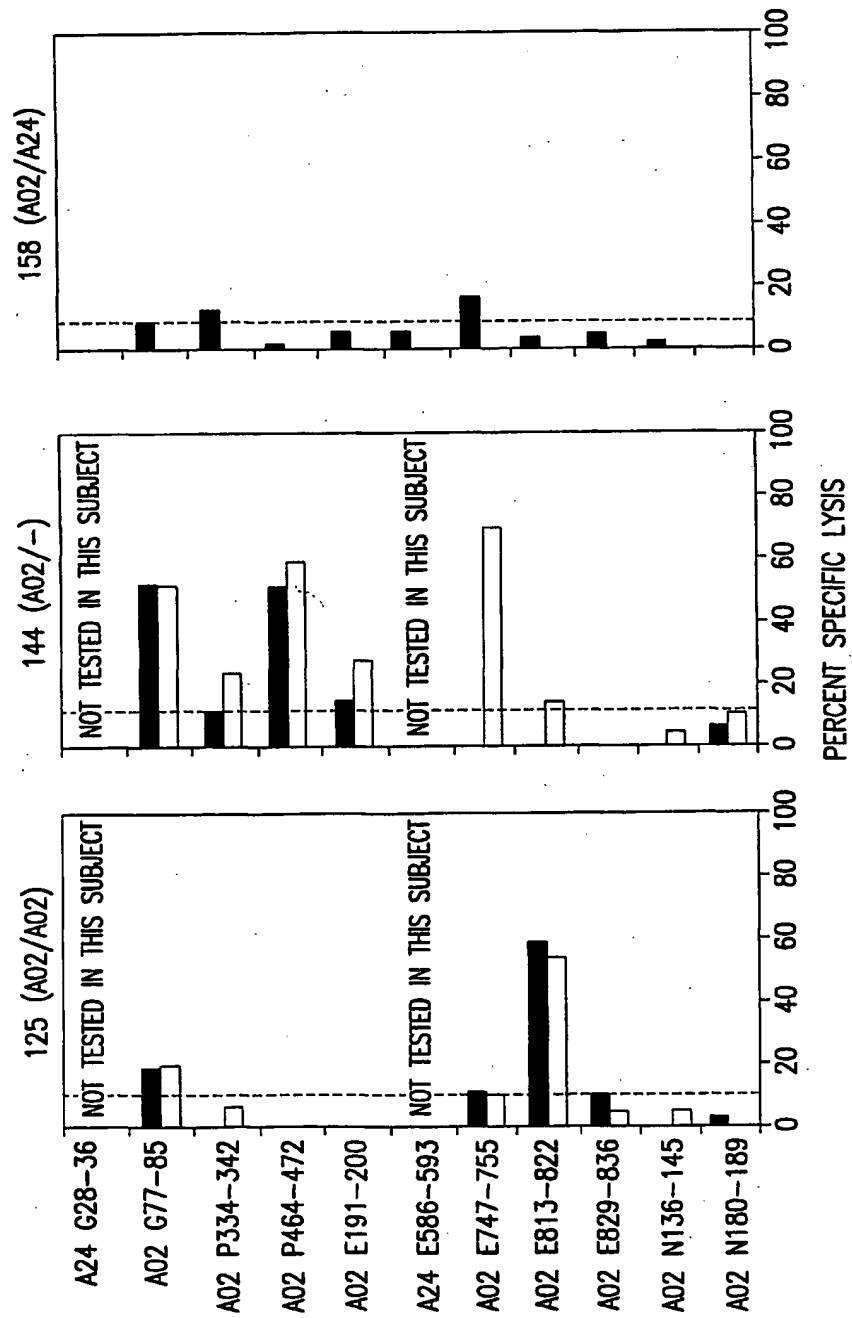


FIG.1D



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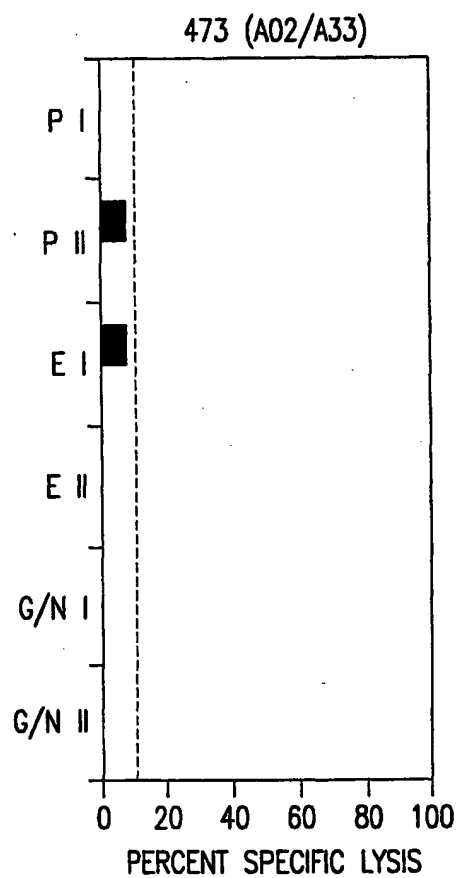


FIG.1E

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